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THE

BIOCHEMICAL JOURNAL

EDITED FOR THE BIOCHEMICAL SOCIETY

BY

W. M. BAYLISS, F.R.S.

AND

ARTHUR HARDEN, F.R.S.

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I. THE EXCRETION OF ANTIGEN.

By AGNES ELLEN PORTER.

From the Physiological Department, Edinburgh University, the Lister Institute, London, and the Royal College of Physicians, Edinburgh.

(*Received January 13th, 1915.*)

If egg-white is introduced parenterally into a rabbit, an albuminuria follows. At the same time the animal thus treated becomes immunised. It produces precipitins, which enable its serum to precipitate in the presence of the protein with which it had been originally injected. In this connection the protein is known as antigen. The combination of antigen and antiserum is not only characterised by a precipitate, it possesses also the faculty of binding the complement of fresh normal serum. The withdrawal of complement is tested for by adding sensitised red blood corpuscles, which, in the absence of free complement, do not become haemolysed.

Hamburger [1902] found that the albuminuria which follows injection of egg-white diminished with each re-injection, as the animal became more immune. Oppenheimer [1903] thought the excretion of albumin irregular in amount, bearing no relation to the degree of immunity or to the formation of precipitins. Cramer [1908] investigated this question, and found that the albuminuria did diminish with further injections of egg-white, but that the amount of albumin excreted depended still more on the stage of digestion. A fasting animal did not make use of the albumin injected into the peritoneal cavity as well as a digesting animal, unless it had received a previous injection of saline solution to induce a local leucocytosis. If the leucocytes took up the protein it was digested by the leucocytic proteolytic ferments and assimilated. Failing a local leucocytosis, it was not assimilated to the same extent and was excreted in much greater amount through the kidney cells. The amount excreted was always less than the amount injected. Cramer also examined chemically the albumin in the urine, and found that it gave every

characteristic chemical test of albumin and that in its behaviour to alcohol and ether it resembled egg-albumin and not serum-albumin. Yet it differed from the original egg-albumin in that it was incapable of forming a precipitate with anti-egg serum. Moreover, it did not give a precipitate with anti-rabbit serum. It was apparently egg-albumin which had lost its "species specificity."

There are two ways in which egg protein may be tested for specificity, first, in vitro, by precipitating with anti-egg serum, secondly, in vivo, by producing precipitin in the serum of the animal into which it has been injected. At Dr Cramer's suggestion, I took up this question in order to determine whether this excreted albumin was still antigen in the sense of being capable of producing antibody when injected into another rabbit, also whether, when mixed with anti-egg serum, it could bind complement.

The Excreted Albumin Tested as Antigen in Vivo.

The albumin was salted out of the urine by saturation with ammonium sulphate, was redissolved and reprecipitated, and was finally dialysed in parchment sacs to remove the precipitating agent. In spite of this treatment it retained a considerable amount of urinary pigment. The urine used for this purpose was that from Rabbit 1 of Group 1, taken on the first and second days after each of four successive injections of egg-white; its behaviour as antigen in vitro will be discussed later. After dialysis, this excreted albumin was thrice injected intraperitoneally into one rabbit, at five-day intervals. On the third occasion the injection was followed within a few minutes by convulsions and death, with all the appearance of anaphylaxis. Blood was taken from the heart immediately after death, and the serum separated from it contained no precipitin. A control rabbit, injected with egg-white which had been similarly precipitated with ammonium sulphate, and then dialysed, did produce precipitin, but of a very low titre, 1/100.

The Excreted Albumin Tested as Antigen in Vitro.

An investigation into the influence of excreted albumin upon anti-egg serum in vitro disclosed, in the first group of rabbits examined, a curious phenomenon.

Three groups of rabbits were used, each group in a different year, season of the year, and laboratory, under different feeding conditions; different

samples of serum were also used in the three groups of experiments. It is interesting that each group of rabbits excreted an albumin which differed from the albumin excreted in the other two groups in its properties as antigen.

Group 1 comprised three rabbits, four times injected, yielding twelve samples of excreted albumin. These samples all possessed the same properties as antigen. They failed completely to precipitate with anti-egg serum. When mixed with anti-egg serum, they exhibited the curious phenomenon of completely inhibiting it from precipitating with fresh egg-white. Although the mixture of excreted albumin and anti-egg serum did not precipitate, it bound complement.

Group 2 consisted of three rabbits, four times injected, yielding twelve samples of excreted albumin. These all failed to precipitate with anti-egg serum, but only one of them exerted any inhibitory influence at all (and that only partial) on precipitation between anti-egg serum and fresh egg-white. They also were unable to bind complement.

Group 3 was represented only by one rabbit, once injected. This sample of excreted albumin precipitated with anti-egg serum, and, with it, bound complement.

The animals were placed in cages, and their urine tested as to quantity, reaction, percentage of urea, and freedom from albumin. They then received an intraperitoneal injection of 10 to 13 cc. egg-white. The urine of the first and second days after injection was examined similarly for quantity, reaction, urea, and albumin. The urine seldom contained more than a trace of albumin after the second day after each injection. The rabbits of the first group were investigated in very cold weather. They were small, about 1·5 kilos. in weight, were poorly fed on bran and water, and showed suppression of urine for 12 to 24 hours after injection. Those of the second group were investigated in hot weather, they were large rabbits above 2·5 kilos. in weight, and were well fed on corn and carrots. No consistent alteration could be observed in the reaction or quantity of urine passed during the day after injection, though there was a rise in the percentage of urea constantly present. The single rabbit representing Group 3 was investigated in spring, it weighed 2 kilos., was fed on corn and turnips, and the urine was increased in total quantity on the day after injection. On the day before injection it was as low as 120 cc., the day after injection 225 cc., the second day after, it rose to 375 cc.

Table from Group 1 showing Inhibition.

(By "immune" urine is meant the albuminous urine of a rabbit which had been injected with egg-white.)

			Precipitate
1.	0·2 cc. Anti-egg serum	+ 0·2 cc. Normal urine 1	0
2.	„ „	+ „ Immune urine 1	0
3.	„ „	+ „ 0·85 % NaCl	0
4.	„ „	+ „ 1/10 Egg-white in NaCl	++
5.	„ „	+ „ „ „ normal urine	++
6.	„ „	+ „ „ „ immune „	+
7.	„ „	+ „ NaCl + 0·2 cc. 1/10 Egg-white	++
8.	„ „	+ „ Normal urine + „ „ „	++
9.	„ „	+ „ Immune „ + „ „ „	0
10.	„ „	+ „ NaCl + „ 1/100 „	++
11.	„ „	+ „ Normal urine + „ „ „	++
12.	„ „	+ „ Immune „ + „ „ „	0

In order to obtain this inhibition it was necessary to add the urine first to the antiserum, before the fresh egg-white (see Exp. 6). If excreted albumin and fresh egg-white were added together, as where egg-white was dissolved 1/10 in the immune (already albuminous) urine (Exp. 9), the inhibition was only partial.

Complement Binding Experiment.

(Two drops of fresh guinea pig serum were added from a capillary pipette to a series of tubes containing antiserum, and NaCl, urine, or egg-white. After one hour at room temperature 0·5 cc. red corpuscles 2 % were added with immune body. The tubes were then placed at 37° for one hour.)

			Haemolysis
1.	0·2 cc. Antiserum	+ 0·2 cc. Normal urine	complete
2.	„ „	+ „ Immune „	trace
3.	„ „	+ „ 0·85 % NaCl	complete
4.	„ „	+ „ Normal urine + 0·2 cc. 1/10 Egg-white	0
5.	„ „	+ „ Immune „ + „ „	0
6.	„ „	+ „ NaCl + „ „	0
7.	0·4 cc. NaCl	+ „ „	complete

Table from Group 2 showing Indifference.

Urine "Rabbit 2" of Group 2 exhibited after the first injection a partial inhibition which was not observed after later re-injections. As it was atypical of the group, the results obtained with urine from Rabbit 1 of the group will be represented.

			Precipitate
1.	0·2 cc. Anti-egg serum	+ 0·2 cc. Normal urine 1	0
2.	„ „	+ „ Immune urine 1	0
3.	„ „	+ „ 0·85 % NaCl	0
4.	„ „	+ „ Normal urine + 0·2 cc. 1/10 Egg-white	++
5.	„ „	+ „ Immune „ + „ „ „	++
6.	„ „	+ „ NaCl + „ „ „	++
7.	„ „	+ „ Normal urine + „ 1/100 „	++
8.	„ „	+ „ Immune „ + „ „ „	++
9.	„ „	+ „ NaCl + „ „ „	++

Complement Binding Experiment.

(Method as above.)

					Haemolysis
1.	0·2 cc. Antiserum	+	0·2 cc.	Normal urine	complete
2.	„ „	+	„	Immune „	„
3.	„ „	+	„	0·85 % NaCl	„
4.	„ „	+	„	Normal urine + 0·2 cc. 1/10 Egg-white	0
5.	„ „	+	„	Immune „ + „ „	0
6.	„ „	+	„	0·85 % NaCl + „ „	0

Table from Group 3 showing Unimpaired Antigen.

					Precipitate
1.	0·2 cc. Antiserum	+	0·2 cc.	Normal urine	0
2.	„ „	+	„	Immune „	++
3.	„ „	+	„	0·85 % NaCl	0
4.	„ „	+	„	Normal urine + 0·2 cc. 1/10 Egg-white	++
5.	„ „	+	„	Immune „ + „ „	++
6.	„ „	+	„	0·85 % NaCl + „ „	++
7.	„ „	+	„	Normal urine + „ 1/100 „	++
8.	„ „	+	„	Immune „ + „ „	++
9.	„ „	+	„	0·85 % NaCl + „ „	++

Complement Binding Experiment.

(Method as above.)

					Haemolysis
1.	0·2 cc. Antiserum	+	0·2 cc.	Normal urine	complete
2.	„ „	+	„	Immune „	0
3.	„ „	+	„	0·85 % NaCl	complete
4.	„ „	+	„	Normal urine + 0·2 cc. 1/10 Egg-white	0
5.	„ „	+	„	Immune „ + „ „	0
6.	„ „	+	„	0·85 % NaCl + „ „	0

These experiments indicate that albumin may be excreted by the kidneys, after intraperitoneal injection of egg-white, in three conditions, as regards the precipitin reaction. In Group 3 its antigen properties were unimpaired in every respect. In Group 1 while chemical properties were retained, the power of precipitating was lost; in view of the inhibition, the albumin may be described as having retained its haptophore affinities. In Group 2 chemical reactions being again unchanged, both precipitating and haptophore properties were lost. It is no easy matter to explain the occurrence of these three conditions of albumin in urine. Michaelis [1903] states that an excess of antigen inhibits the precipitation, though Chapman [1910] finds that excess of antigen does not affect the reaction in either direction. In any case this explanation does not meet the present case. The inhibition was sometimes exerted by urine containing only a trace of albumin, and not by

urine containing much. Also, as has been already stated, fresh egg-white, dissolved in albuminous and inhibitive urine, while increasing the total protein present, diminished the inhibition, which was always greater where the inhibiting urine was added first to the antiserum than where both forms of protein were added together.

Another possible explanation of the loss of precipitating power, and of acquisition of inhibitory influence might lie in the action of the urea. Limbourg [1887] found that urea has a very powerful solvent action upon protein. Spiro [1900] observed that fibrin and coagulated egg-white were dissolved by urea at 37°, and could not be recoagulated by the application of heat as long as the urea was present. If however the solution of fibrin in urea was dialysed and the urea thereby removed the coagulability of the protein returned. Pick [1902] found that saturated urea solution (50 % in water) prevents the precipitin reaction through its action upon the serum protein. This fact I am able to confirm in the concentration, 50 %, which Pick used. I could find, on the other hand, no influence whatever in the case of 5 % urea. Egg-white dissolved in 0·85 % salt solution containing 5 % urea, did not lose as antigen in the slightest degree. If 5 % urea in salt solution was added to anti-egg serum in equal proportions no inhibition to the action of egg-white could be detected, other than by diluting the serum by one-half, an action in which it was equalled by control salt solution. It may be stated that although the percentage of urea in the urine rose after injection, it on no occasion rose as high as 0·5 %. It is possible that albumin may be subjected to a high percentage of urea in the kidney tubules, especially where suppression of urine occurs. Egg-white was therefore treated with an equal quantity of saturated urea solution at 0°, at room temperature, at 37° for 24 hours, and at 56° for two hours. The egg-white was then diluted to 1/10 in salt solution, making 1/10 egg-white in 5 % urea solution. No impairment of antigen properties could be detected. Egg-white diluted 1/10 in saturated urea solution, and after 24 hours treatment diluted to 1/100 in salt solution, did not appear to differ in any way from the control experiment with 1/100 urea solution. This result agrees with Spiro's observation that the solvent action of urea does not consist in any permanent change in the protein; if the urea is removed either by dialysis or dilution to an innocuous concentration, the solvent action disappears. This makes it clear that the action of urea in the kidney tubules is not responsible for the change in the albumin excreted in the urine. The concentration of the urea passed, under 0·5 %, was too low to inhibit precipitation.

The urine normally contains pepsin and trypsin [Sahli, 1885]. Pepsin and trypsin act for some time on protein changing its physical condition before a point is reached at which chemical protein reactions are changed or lost. Klug [1902] has found for pepsin, and Roberts [Oppenheimer, 1909] for trypsin, that increase of temperature, at least between such limits as 0° and 40°, has an accelerating influence upon protein digestion. Now the albumin of Group 2, where the antigen was most affected, was excreted in very hot weather. On the other hand, the weather was warmer in the third case (in spring), where the albumin was unimpaired, than in the first, where the albumin had lost its precipitating power, and where there was much cold and frost. It must be mentioned that toluene and thymol were placed in the urine bottles.

The first step in protein digestion is the most difficult. This means that protein digestion is unlikely to take place in "layers"; that is to say, when a part of the protein present has reached a certain stage, the succeeding stages are rapid, so that all stages of protein digestion may be present coincidently.

I treated egg-white with 0·3 % trypsin in N/100 NaOH, and found that this enzyme had little or no action in 24 hours even in the presence of urea on antigen properties. Pepsin had a far greater action. I used 1/500 Ray's pepsin in N/50 acid, testing the treated egg-white in 0·5 hour, 1 hour, 1·5 hours, etc., after treatment at 37°, and room temperature. The antigen properties were quickly weakened, but a trace of precipitating power was left until the chemical reactions for albumin had also disappeared. When the egg-white was shown by chemical tests to be largely digested, the precipitating power was almost gone.

From these results it appears improbable that the deterioration of specific egg-white can be ascribed either to the action of the urea, or of pepsin and trypsin present.

. Haswell Wilson [1909] has found that albumin excreted in human urine in disease is constant in its action as antigen in the complement binding test, but he was dealing with diseased kidneys, whereas in the rabbits under investigation the kidneys were normal. I am unable to find an explanation of the change of specificity in this albumin, other than that of a physical nature, such as Cramer has indicated. Cramer points out that these results are incompatible with the generally accepted view that the power of protein to act as antigen is dependent on a specific chemical group in the protein molecule. These facts, according to him, make it necessary to abandon this view and to assume that the property of protein to act as antigen either

depends on the physical state of the protein molecule, or that it is due to a substance which is always associated with protein in protoplasm without forming part of the protein molecule, and which can be separated from it without altering the purely chemical properties of the protein.

This research was partly carried out with the help of a Lister and a Carnegie Research Scholarship.

Conclusions.

Egg-white introduced parenterally into a rabbit is excreted unchanged in chemical properties, but may be in three possible variations as regards specificity:

1. It may retain partial properties as antigen, binding complement in the presence of anti-egg serum, not causing precipitation but capable of exercising an influence over antibody so as to prevent it precipitating with fresh antigen.

2. It may be excreted in a non-specific form completely indifferent as antigen.

3. It may retain full antigen properties.

This variation in specificity of excreted albumin points to a physical rather than a chemical explanation of the precipitin reaction.

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II. THE TOXICITY OF SODIUM PYROPHOSPHATE ADMINISTERED IN FOOD; WITH A NOTE ON TOXIC COTTON SEED MEAL.

By WILLIAM LEGGE SYMES and JOHN ADDYMAN GARDNER.

From the Physiological Laboratory of the University of London, South Kensington.

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Our attention was drawn to the comparative physiological activity of ortho- and pyrophosphate of sodium in the course of observations on the physiological effects of some camphene-phosphonates, which appeared to bear to one another the same relation as that which exists between the phosphates in question.

In the course of this work [1911] we compared the activity of the phosphates, in solution, by intravenous injection, and confirmed the findings of earlier workers, viz., that the orthophosphate is relatively inert, whilst the pyrophosphate is much more toxic.

We attributed the activity of the pyrophosphate, in part, to the alkalinity of its solutions, in part to its acid radicle. Its solutions after "neutralisation," with hydrochloric acid, to litmus, to phenolphthalein, and to methyl orange, though less active, were still markedly toxic. Almost simultaneously with the above, Starkenstein [1911] referred the toxicity of pyrophosphate solutions, in part to their anions, in part to their kations. He further stated, in agreement with Gamgee, Priestley, and Larmuth [1877], that pyrophosphate administered by the mouth was not poisonous.

Crawford [1910] on the other hand, administered sodium pyrophosphate to rabbits, by the mouth, with fatal results. The salt, given in doses of 0·5–1·0 g. dissolved in 25–40 cc. of water, was usually lethal within three days, the post-mortem appearances described by him, suggesting that the deaths were due to an irritant poison.

It seemed therefore worth while to test afresh the results of administering the salt by the alimentary canal. This has been done on rabbits, cats, and rats, with the result that large doses, administered for prolonged periods, have failed to produce acute poisoning.

A sheep, on the other hand, died after taking 21 grams of the salt.

In this, as in our earlier paper, the chemical side has been the work of Gardner, whilst the physiological portion is that of Symes.

Observations on Rabbits.

The first set of observations was made on three pairs of young rabbits, fed with the same food, viz., oats, bran, and chopped cabbage.

The first and second pairs received daily doses, of ortho- and of pyro-phosphate of sodium respectively, whilst the third was kept to control the effect of outside factors. In the first two cases (pairs) the salt was powdered, mixed with 15 g. of chopped cabbage, and given to each animal separately. This ensured complete ingestion of the salt by each individual, as the animals devoured the cabbage with relish. Apart from the above the animals were fed, and housed, in pairs. They were weighed, singly, at the commencement of the experiment, and at intervals of seven days during its continuance. Table I shows the result of four weeks of this régime. The doses were doubled each week and, molecule for molecule, that of the orthophosphate was much the greater.

TABLE I.

Body-weights (weekly) of young rabbits, fed on a mixed diet, with and without addition of phosphate.

	On Na_2HPO_4 12 Aq.		Daily dose per head	On $Na_4P_2O_7$ 10 Aq.		Daily dose per head	No added phosphate	
Week	Rabbit A	Rabbit B		Rabbit C	Rabbit D		Rabbit E	Rabbit F
	Kg.	Kg.	g.	Kg.	Kg.	g.	Kg.	Kg.
0	0·90	0·90	—	0·80	0·60	—	1·10	0·60
1	1·10	1·00	0·32	0·98	0·80	·13	1·40	0·82
2	1·10	1·05	0·64	1·00	0·77	·26	1·40	0·80
3	1·15	1·10	1·3	1·10	0·70	·52	1·45	0·90
4	1·20	1·10	2·6	1·00	0·80	1·0	1·70	1·00
Increase in body-weight	33 %	22 %		25 %	33 %		55 %	67 %

The above figures suggest that, although neither phosphate proved acutely poisonous, each one had, at this stage of life, an adverse influence on growth. This influence was not maintained in later observations on the same animals.

On completion of these observations the animals were all kept on the same mixed diet, without phosphate, for eight weeks. During this period their weights increased as follows:

Rabbit	A	from	1·20 kg.	to	1·75 kg. = 46 %
	B		1·10		1·35 = 23
	C		1·00		1·60 = 60
	D		0·80		0·95 = 19
	E		1·70		1·80 = 6
	F		1·00		1·35 = 35

The growth of rabbit D during this period is, for its weight, strikingly small. This animal was, from the commencement, weakly, and light in proportion to its apparent bulk, and its subsequent death did not appear to be primarily due to the experiment.

After this interval (eight weeks), the animals were again treated as shown in Table I, with the results given in Table II.

TABLE II.

Weekly body-weights of rabbits as in Table I.

	On Na_2HPO_4 12 Aq.		Daily dose	On $Na_4P_2O_7$ 10 Aq.		Daily dose	No added phosphate	
Week	Rabbit A	Rabbit B	per head	Rabbit C	Rabbit D	per head	Rabbit E	Rabbit F
	Kg.	Kg.	g.	Kg.	Kg.	g.	Kg.	Kg.
0	1·75	1·35	—	1·60	0·95	—	1·80	1·35
1	1·70	1·70	0·35	1·60	0·80	0·23	1·80	1·30
2	1·80	1·50	0·71	1·80	1·00	0·46	1·90	1·35
3	2·10	1·70	1·4	1·90	1·30	0·93	1·85	1·50
4	2·30	1·70	2·8	1·90	1·45	1·9	2·00	1·70
5	2·30	1·85	„	2·00	1·50	„	2·20	1·80
6	2·40	2·00	„	2·00	1·60	„	2·15	1·90
7	2·45	1·90	„	2·15	1·50	„	2·20	1·95
8	2·50	1·90	„	2·30	1·60	„	2·10	2·00
9	2·70	2·30	„	2·20	1·50	„	2·15	2·00
10	2·65	2·20	„	2·20	1·20	„	2·25	2·20
11	2·60	2·10	1·8	2·15	1·10	1·2	2·30	2·25
12	2·70	2·20	„	2·20	1·10	„	2·25	2·20
Increase in body-wt.	54 %	63 %		38 %	16 %		25 %	63 %

The doses were doubled each week for the first month. That of the orthophosphate was, again, the greater.

Apart from Rabbit D, these figures do not suggest that either phosphate had, with the older rabbits, any adverse effect on growth. This animal which, as above stated, had appeared weakly from the beginning, died about a week after the observations were completed.

The autopsy revealed no gastric or intestinal lesion, nor anything to show that the death resulted from the pyrophosphate.

Towards the end of this series one rabbit of each group was isolated, for one week, for collection of its urine and faeces.

In each case the salt was eaten in full. On estimating the P_2O_5 in the dejecta, by Neumann's method, the whole of the P_2O_5 given, as orthophosphate, was recovered; whilst the P_2O_5 recovered from the urine and faeces of the rabbit taking 2·3 g. P_2O_5 as pyrophosphate only slightly exceeded that from the control animal.

As already mentioned, the whole of the pyrophosphate was eaten. The missing P_2O_5 was not retained in the animal, but deposited as "earthy phosphate" on the floor of the cage, from which it was not dislodged by the water with which the cage was sluiced at the end of each week, but was subsequently, in great part, recovered by washing with hydrochloric acid.

Two further experiments yielded the same result, i.e. all the P_2O_5 given as Na_2HPO_4 was recovered from the dejecta plus water washings; whilst much of that given in $Na_4P_2O_7$ was found as an almost invisible deposit on the floor of the cage.

On this account, the experiment was repeated on a cat, in the anticipation that the high acidity of its urine would diminish or prevent the deposition of the phosphates.

Observations on Cats.

The results on two cats were as follows.

The first, a full grown but rather thin cat, was given (and ate) 2 g. of $Na_4P_2O_7$, 10 aq., daily, for 15 days, mixed with soaked biscuit and milk. The animal showed no sign of discomfort and gained 100 g. in weight during this period, in spite of slight diarrhoea.

The second, a younger cat, was given (and ate) the same dose for 13 days. This animal vomited once, but gained 100 g. in weight during the observation. The same cat, two months later, took 2 g. of Na_2HPO_4, 12 aq., daily, for 9 days, also without any symptom of acute poisoning. Both phosphates produced slight diuresis, and some tendency to diarrhoea. In consequence of the latter, the faeces were not collected. The urine, collected during a week of each régime, in comparison with that of a week on the same diet without addition of phosphate yielded P_2O_5 as shown in the following table.

TABLE III.

Urine of a cat on a mixed diet, and on the same diet plus phosphate.

Régime	Volume (one week)	Sp. Gr.	P_2O_5 recovered	P_2O_5 given as salt	Difference between P_2O_5 recovered, and P_2O_5 given as salt
Control	748 cc.	1026	2·0 g.	0·0 g.	2·0 g.
Orthophosphate	819 cc.	1040	4·7 g.	2·4 g.	2·3 g.
Pyrophosphate	776 cc.	1038	5·5 g.	3·8 g.	1·7 g.

It is obvious that both phosphates were absorbed, and that loss by deposit was not great.

Observations on Rats.

A series of observations was made on three pairs of rats. The animals were fed on bread moistened with water and on oats. The first pair was given, daily, with the soaked bread, 0·5 g. of Na_2HPO_4, 12 aq.; the second pair an equal weight of $Na_4P_2O_7$, 10 aq.; whilst the third pair had no phosphate added to its ration. The animals were fed in pairs, and weighed (also in pairs) once a week. Table IV shows the body-weights under nine weeks of this régime.

TABLE IV.

Body-weights (weekly) of pairs of white rats, fed on a mixed diet, with and without addition of phosphate.

Week	On Na_2HPO_4 12 Aq. 0·5 g. daily	On $Na_4P_2O_7$ 10 Aq. 0·5 g. daily	No added phosphate
0	217 g.	175 g.	229 g.
1	229 „	193 „	233 „
2	232 „	197 „	248 „
3	235 „	192 „	254 „
4	238 „	206 „	267 „
5	244 „	213 „	263 „
6	259 „	224 „	263 „
7	256 „	220 „	273 „
8	256 „	222 „	277 „
9	245 „	219 „	285 „
Increase in body-weight	13 %	25 %	25 %

The pyrophosphate pair, starting 25 % below the controls, grew at the same nett rate as these, whilst the orthophosphate pair starting at the same level (as the controls) grew less rapidly. Both of the phosphate pairs lost weight after the sixth week.

It was noted that the pyrophosphate ration was eaten with less relish than the others, and that on 21 days (out of the 63) some of it was left, thereby entailing loss of some of the phosphate. But since on the remaining 42 days the dose was fully ingested, the table confirms the indications of the earlier series, that neither phosphate was acutely poisonous.

Observations on a Sheep.

On account of the supposed presence, and toxicity, of pyrophosphate in some samples of cotton seed meal, it was thought well to test the effect of pyrophosphate on a sheep, and we are indebted to the Director of the Lister Institute for affording us facilities for this portion of the work.

A full grown wether was given, daily, 1·0 g. of $Na_4P_2O_7$, 10 aq., in 50 g. of dry oatmeal, in addition to hay, for a week. The meal was eaten with relish and produced no noticeable effect. After a week's interval, the daily dose of pyrophosphate was recommenced and doubled. This was still taken with relish, but produced some thirst and restlessness, and the animal was found dead on the morning following the seventh dose, i.e. after a total intake of 21 g. of pyrophosphate.

The autopsy showed marked softening of the mucous membrane of the rumen and a small patch of inflammation, in its wall, opposite the spleen, to which it was firmly adherent.

The reticulum and omasum appeared normal, whilst the abomasum and small intestine were inflamed throughout. No ulcer was seen in any portion of stomach or intestine. This result agrees with those of Crawford, on rabbits, in which the conditions of experiment were substantially those in the case of the sheep. Crawford administered $Na_4P_2O_7$ in (approximately 2 %) solution, i.e. he introduced a strongly alkaline solution into the stomachs of his animals.

Introduction of dry $Na_4P_2O_7$ into the already alkaline contents of the sheep's rumen yields, likewise, a strongly alkaline solution. This alkaline solution produced the same effect as Crawford's more concentrated alkaline solutions, though without visible ulceration and less rapidly.

To our rabbits, the pyrophosphate was given as powder in chopped cabbage; to the rats and cats, as powder in moistened bread and biscuit respectively. In these cases, the salt was presumably, in great part, (if not wholly) neutralised as rapidly as it reached the stomach wall. It certainly produced no acute poisoning.

Equally certainly it was largely absorbed, as shown by the analyses of

the urines, and urinary deposits. It would appear, therefore, either that the pyrophosphoric acid, liberated by the gastric juice, was hydrolysed to the less active orthophosphoric acid (as suggested by Starkenstein), or that (as supposed by Gamgee) the absorption of pyrophosphate was balanced by its prompt excretion.

Note on Toxic Cotton Seed Meal.

In relation to cotton seed poisoning, mentioned above, we may add that a sample of toxic cotton seed meal which has come into our hands, has proved fatal to rats. Four of these animals, fed exclusively (and *ad libitum*) on the meal, died on the 5th, 7th, 13th and 14th days respectively, showing gastro-intestinal inflammation without visible ulceration or noticeable softening of the mucous membrane. The appearances were, in short, those common to irritant poisons, not distinctive of any particular one.

As detailed above, our rats consumed, daily, 0·5 g. per head of crystalline sodium pyrophosphate (= 0·3 g. $Na_4P_2O_7$), for nine weeks, without visible harm. On the other hand, four rats fed on oats, and bread, plus 5 g. of cotton seed meal per head, died on the 10th, 15th, 24th, and 25th days respectively. Four other rats fed on oats, bran, and 5 g. per head of the same cotton seed meal, after previous extraction with alcohol and with ether, lived 26, 31, 40, and 50 days respectively; whilst four (control) rats, fed on oats and bread only, maintained their health and gained 77 g. in weight, in the 50 days of the experiment. The P_2O_5 content of the (air dry) cotton seed meal was 0·43 %, and amounted to 0·022 g. in the daily ration. This corresponds to 0·07 g. of $Na_4P_2O_7$, 10 aq., i.e. less than one-seventh of the quantity tolerated, daily, for nine weeks. It is clear from the reduction of toxicity by alcohol and ether, and from the low phosphate content of the seeds that sodium pyrophosphate cannot be the chief poison in the meal in question.

It was hoped to identify the poisonous principles and to extend the observations to ruminants, but the non-renewal of a small grant from the Board of Agriculture has rendered this, for the present, impracticable.

Summary and Conclusions.

Sodium pyrophosphate administered, with food, to rabbits, cats, and rats, is devoid of the toxic action that it shows when intravenously injected. This confirms the verdict of Gamgee and his pupils, and of Starkenstein.

Administered to a sheep, in the same way, it has proved lethal, producing effects similar to those described, by Crawford, as following its administration, in aqueous solution, to rabbits.

Such toxic action as sodium pyrophosphate exerts, when administered by the mouth, differs from that of the same compound, intravenously injected, in that it is wholly due to the alkalinity of the salt and not to the acid radicle.

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III. NOUVELLES RECHERCHES SUR LA DIGESTION DES PROTÉINES DE LA VIANDE CUITE CHEZ LE CHIEN (SECONDE COMMUNICATION).

PAR EDGARD ZUNZ.

(*Institut de Thérapeutique de l'Université de Bruxelles.*)

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I. INTRODUCTION.

Dans un travail antérieur [1913], j'ai utilisé la centrifugation pour séparer, chez le chien, les protéines et leurs diverses espèces de dérivés dans le contenu tant de chacune des deux portions principales de l'estomac que de la première portion de l'intestin grêle à divers moments après l'ingestion de viande cuite de cheval. J'ai ainsi comparé la répartition de l'azote entre les protéines intactes, l'acidalbumine, les protéoses et les autres dérivés des substances albuminoïdes, selon qu'on emploie pour cette séparation l'ancienne méthode par filtration ou le nouveau procédé par centrifugation. Ce dernier permet d'éviter les modifications dans la composition des filtrats, signalées par *Christiansen* [1912].

Après l'ingestion de viande de cheval, on trouve en moyenne une proportion un peu plus considérable d'acidalbumine et de protéoses considérées ensemble, et par conséquent une proportion un peu moindre des autres produits de désintégration des protéines dans les diverses régions du tube digestif lorsqu'on emploie la centrifugation que lorsqu'on utilise la filtration. Il semble que pendant le laps de temps assez long exigé parfois par les filtrations successives nécessitées par la détermination de la répartition de l'azote entre les protéines et leurs diverses espèces de dérivés, une partie, relativement faible il est vrai, de l'acidalbumine et des protéoses se transforme en peptones et en substances abiurétiques. Il se peut aussi que les filtres retiennent par adsorption plus facilement l'acidalbumine et les protéoses que les peptones et les dérivés abiurétiques des protéines.

Quoi qu'il en soit, on doit renoncer à la méthode par filtration et lui préférer celle, plus rapide et plus exacte, par centrifugation. A coup sûr, bien que les différences entre les résultats obtenus par les deux procédés ne soient pas très considérables pour ce qui concerne, chez le chien, la digestion de la viande cuite de cheval, elles méritent néanmoins d'appeler l'attention. Aussi m'a t'il paru utile d'appliquer la méthode par centrifugation à l'étude de la digestion par le chien de la viande cuite de bœuf, afin de voir si les différences signalées ci-dessus entre les résultats des deux procédés à propos de la viande cuite de cheval se vérifiaient pour celle de bœuf.

J'ai, en outre, profité de ces expériences pour rechercher la teneur en azote ammoniacal et en azote aminé des diverses régions du tube digestif au cours de la digestion de la viande cuite de bœuf.

II. Technique.

On a débarrassé les chiens au préalable des vers intestinaux [Zunz, 1910, 1]. On a maintenu ces animaux 24 à 36 heures à jeûn, tout en leur permettant de boire de l'eau à volonté. On leur a ensuite donné 25 grammes de viande cuite de bœuf par kilogramme de poids. On a déterminé, par la méthode de *Kjeldahl*, la teneur en azote de chaque échantillon de viande après dissolution dans l'acide sulfurique.

On a sacrifié les 12 chiens A à L, 1 à 12 heures après le repas, par piqûre du bulbe et saignée des carotides. On a rapidement incisé la paroi abdominale en ayant soin de ne pas bouger aux viscères. On a isolé, par le procédé que j'ai décrit [1910, 1], les deux régions principales de l'estomac et la première portion de l'intestin grêle.

Dans mes communications antérieures j'ai conservé aux deux parties essentielles de l'estomac du chien les dénominations de fundus et de région ou d'antre prépylorique, adoptées par beaucoup de physiologistes. Mais si l'on considère les travaux les plus récents des anatomistes et des radiologues, il vaut mieux appeler corps de l'estomac la portion de cet organe désignée jusqu'à présent sous le nom de fundus; elle comprend le corpus gastricus, le canalis gastricus et le fundus proprement dit ou sac cardiaque. Quant à la portion prépylorique, elle correspond à la fois à l'antrum pyloricum et au vestibulum pyloricum des anatomistes.

On a recueilli séparément dans de l'eau distillée le contenu du corps de l'estomac, celui de la portion prépylorique de cet organe et celui de la première partie de l'intestin grêle. On a aussitôt soumis ces bouillies alimentaires

à l'ébullition, après avoir ajouté le cas échéant de l'acide chlorhydrique dilué au contenu intestinal afin de le rendre légèrement acide, puis on les a laissé refroidir.

On a séparé, par une centrifugation de 10 à 15 minutes, à la centrifugeuse Jouan, tournant à la vitesse de 7000 tours par minute, les matières non attaquées et les protéines coagulées du liquide contenant l'azote soluble. On a remis le précipité en suspension dans de l'eau distillée et l'on a procédé à une nouvelle centrifugation. On a répété ces deux opérations quatre à six fois, c'est-à-dire jusqu'au moment où le liquide surnageant n'a plus donné la réaction du biuret.

Tant pour le contenu du corps de l'estomac que pour celui de la portion prépylorique de cet organe, on a traité par l'acide sulfurique la partie non dissoute du bol alimentaire, et l'on a dosé, par la méthode de *Kjeldahl*, l'azote coagulable de ces solutions.

On a réduit, le cas échéant dans le vide, à une température ne dépassant pas 35 à 40° C., les liquides surnageants et les eaux de lavage renfermant l'azote soluble non coagulable, de manière à obtenir, pour le contenu de chacune des trois portions du tube digestif examinées, un filtrat initial, dont le volume total atteigne 150 à 200 cm.³ On a prélevé de chaque filtrat initial: 1° trois portions de 5 cm.³ destinées au dosage de l'azote soluble non coagulable par la méthode de *Kjeldahl*; 2° deux portions de 20 cm.³ destinées au dosage de l'ammoniaque par la méthode de *Folin*; 3° deux portions de 10 cm.³ destinées au dosage de l'azote aminé aliphatique par la méthode de *van Slyke*.

On a effectué cette dernière détermination en suivant exactement les indications les plus récentes du savant américain [van Slyke, 1912, 1913]. On s'est servi, dans ce but, du nouvel appareil imaginé par cet auteur afin de permettre une agitation continue de même intensité pendant les cinq minutes d'action de l'acide nitreux, de telle sorte que les conditions de la réaction restent identiques dans tous les cas. J'ai toutefois fait modifier légèrement cet appareil, de façon à augmenter la précision des lectures. La burette renfermant le liquide à analyser est divisée en cinquantièmes de centimètre cube. Les six premiers centimètres cubes de la burette gazométrique sont gradués en vingtièmes de centimètre cube. On a calculé en milligrammes l'azote ammoniacal et l'azote décelable par la méthode de *van Slyke* renfermés dans l'ensemble du filtrat initial. On a soustrait du chiffre ainsi calculé par la méthode de *van Slyke* le tiers de l'azote ammoniacal. Le tableau I indique les chiffres d'azote aminé ainsi corrigés.

J'ai exposé antérieurement les raisons pour lesquelles j'avais adopté cette façon de procéder dans les recherches sur la digestion des protéines de la viande cuite de cheval. Afin de pouvoir comparer les résultats obtenus de cette manière et ceux des nouvelles recherches sur la digestion de la viande cuite de bœuf, j'ai eu soin d'employer la même technique dans ces deux séries d'expériences. La technique plus perfectionnée, basée sur le procédé de désalbuminisation de *van Slyke* et *Meyer* [1912] et sur l'emploi d'uréase de la façon indiquée par *Marshall* [1913], n'est, du reste, pas très aisée à appliquer lorsqu'on doit opérer avec un liquide ne renfermant que très peu d'azote aminé.

Le restant du filtrat renfermant l'azote soluble non coagulable sert à déterminer, par la méthode que j'ai proposée [1910,1], successivement l'azote de l'acidalbumine, l'azote de l'hétéroalbumose et de la protoalbumose[1], l'azote des autres protéoses, l'azote des dérivés des protéines précipités par l'acide phosphotungstique mais pas par le sulfate de zinc, l'azote des composés précipités ni par l'un ni par l'autre de ces réactifs en ayant soin de séparer les précipités successifs par une centrifugation énergique de 10 à 15 minutes, puis de décanter les liquides parfaitement clairs ainsi obtenus.

On a aussi examiné si les liquides débarrassés des protéines, de l'acid-albumine et des protéoses, donnaient encore la réaction du biuret décelant la présence des peptones vraies de *Kühne*.

III. Résultats.

Dix tableaux rassemblent les résultats de ces expériences.

Le tableau I indique 1° la quantité d'azote ingérée, 2° la répartition de l'azote trouvé dans le corps de l'estomac entre les protéines non attaquées et les divers groupes de produits de leur digestion, 3° les quantités d'azote ammoniacal et d'azote aminé aliphatique existant dans cette partie de l'estomac. Le tableau II a trait à la répartition de l'azote de la région prépylorique de cet organe entre les protéines intactes et leurs principaux dérivés ainsi qu'aux quantités totales d'azote ammoniacal et d'azote aminé aliphatique y renfermées. Le tableau III donne ces mêmes indications pour l'ensemble de l'estomac et mentionne, en outre, la quantité d'azote ingéré disparue de cet organe au bout du laps de temps considéré. Pour les chiens H à J, sacrifiés 8 à 10 heures après le repas, on a dû se borner à examiner le contenu de l'estomac total, le corps de l'estomac ne renfermant plus de bol

[1] Ou plutôt des protéoses précipitées par demi-saturation au moyen du sulfate de zinc en milieu acide.

TABLEAU I.

Chien en expérience	Poids de l'animal, en grammes	Durée de la digestion, en heures	Quantité de viande ingérée, en grammes	Azote ingéré, en grammes	Azote trouvé dans le corps de l'estomac																							
					en grammes																				en milligrammes			
					à l'état coagulable		à l'état incoagulable																						
							sous forme de protéoses																						
						sous forme d'acid-albumine	hétéroalbumose et protoalbumose	autres protéoses	total	sous forme d'autres produits																			
										précipités par l'acide phosphotungstique																			
										non précipités par l'acide phosphotungstique																			
										total																		
										total																		
A	4300	1	107·5	3·7237	2·3012	0·0026	0·2742	0·2460	0·3589	0·0132	0·0248	0·0380	0·5608	2·8620	1·03	21·90	—	—	—	—	—	—	—	—	—	—	—	—
B	3200	2	80	2·9952	1·6982	0·0009	0·1708	0·1881	0·5202	0·0167	0·0394	0·0561	0·4159	2·1141	10·35	29·47	—	—	—	—	—	—	—	—	—	—	—	—
C	13200	3	330	11·0385	5·0346	0·0008	0·2025	0·0712	0·2737	0·0270	0·0082	0·0352	0·3097	5·3443	5·90	7·56	—	—	—	—	—	—	—	—	—	—	—	—
D	8600	4	215	8·7290	2·5620	0·0706	0·3820	0·4150	0·7970	0·1397	0·0178	0·1575	1·0251	3·5871	0·29	46·86	—	—	—	—	—	—	—	—	—	—	—	—
E	7500	5	187·5	7·5300	1·1290	0·0015	0·1962	0·6089	0·8051	0·1102	0·0580	0·1682	0·9748	2·1038	2·63	52·76	—	—	—	—	—	—	—	—	—	—	—	—
F	9700	6	242·5	8·0850	0·3334	0·2140	0·3159	0·3202	0·6361	0·0548	0·0887	0·1425	0·9936	1·3270	16·86	16·10	—	—	—	—	—	—	—	—	—	—	—	—
G	12850	7	321·25	13·0620	0·2217	0·0029	0·1869	0·1559	0·3428	0·0082	0·0169	0·0251	0·3708	0·5925	12·04	9·15	—	—	—	—	—	—	—	—	—	—	—	—
H	13500	8	337·5	12·7845	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
I	5700	9	142·5	5·3879	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
J	9300	10	232·5	8·1561	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
K	10200	11	255	9·1339	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
L	9850	12	246·25	8·9079	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLEAU II.

Azote trouvé dans la région prépylorique de l'estomac

Chien en expérience	Durée de la digestion, en heures	en grammes									en milligrammes	
		à l'état coagulable	à l'état incoagulable							total		
			sous forme d'acid-albumine	sous forme de protéoses			sous forme d'autres produits		total			
				hétéroalbumose et protoalbumose	autres protéoses	total	précipités par l'acide phosphotungstique	non précipités par l'acide phosphotungstique	total		à l'état d'azote ammoniacal	à l'état d'azote aminé aliphatique
A	1	0·1326	0·0002	0·0230	0·0247	0·0477	0·0082	0·0196	0·0278	0·0757	0·2083	1·11	4·26
B	2	0·1233	0·0000	0·0155	0·0299	0·0454	0·0016	0·0297	0·0313	0·0767	0·2000	4·52	6·59
C	3	0·1576	0·0002	0·0208	0·0126	0·0334	0·0846	0·0345	0·1191	0·1527	0·3103	5·60	4·78
D	4	0·2358	0·0110	0·0445	0·0674	0·1119	0·0416	0·0201	0·0617	0·1846	0·4204	0·27	9·15
E	5	0·1905	0·0004	0·0056	0·0317	0·0373	0·0304	0·0558	0·0862	0·1239	0·3144	5·05	4·73
F	6	0·0370	0·0323	0·0438	0·0526	0·0964	0·0071	0·0344	0·0415	0·1702	0·2072	6·72	7·60
G	7	0·1786	0·0006	0·0246	0·0544	0·0790	0·0105	0·0359	0·0464	0·1260	0·3046	4·50	11·65

TABLEAU III.

Chien en expérience	Durée de la digestion, en heures	Azote trouvé dans l'estomac total												
		en grammes										en milligrammes		
		à l'état coagulable	à l'état incoagulable								total			
			sous forme d'acid-albumine	sous forme de protéoses			sous forme d'autres produits			total				
				hétéroalbumose et protoalbumose	autres protéoses	total	précipités par l'acide phosphotungstique	non précipités par l'acide phosphotungstique	total			à l'état d'azote ammoniacal	à l'état d'azote aminé aliphatique	Azote disparu de l'estomac, en grammes
A	1	2·4338	0·0028	0·2972	0·2707	0·5679	0·0214	0·0444	0·0658	0·6365	3·0703	2·14	26·16	0·6534
B	2	1·8215	0·0009	0·1863	0·2180	0·4043	0·0183	0·0697	0·0874	0·4926	2·3141	14·87	36·06	0·6851
C	3	5·1912	0·0010	0·2233	0·0838	0·3071	0·1116	0·0427	0·1543	0·4624	5·6546	11·50	12·34	5·3839
D	4	2·7978	0·0816	0·4265	0·4824	0·9089	0·1813	0·0379	0·2192	1·2097	4·0075	0·56	56·01	4·7225
E	5	1·3195	0·0019	0·2018	0·6406	0·8424	0·1406	0·1138	0·2544	1·0987	2·4182	7·68	57·49	5·1118
F	6	0·3704	0·2463	0·3597	0·3728	0·7325	0·0619	0·1231	0·1850	1·1638	1·5342	23·58	23·70	6·5508
G	7	0·4003	0·0035	0·2115	0·2103	0·4218	0·0187	0·0528	0·0715	0·4968	0·8971	16·54	20·80	12·1649
H	8	0·1384	0·0479	0·1676	0·3036	0·4712	0·0352	0·0828	0·1180	0·6371	0·7755	25·76	8·25	12·0090
I	9	0·0108	0·0003	0·0436	0·0892	0·1328	0·0395	0·0238	0·0633	0·1964	0·2072	7·85	4·69	5·1807
J	10	0·0367	0·0068	0·0604	0·0719	0·1323	0·0167	0·0259	0·0426	0·1817	0·2184	4·62	2·28	7·9377

alimentaire ou à peine. Quant aux chiens K et L, sacrifiés respectivement 11 et 12 heures après le repas, leurs estomacs étaient entièrement vides. Le tableau IV relate les chiffres d'azote trouvés chez les chiens A à J dans la première portion de l'intestin grêle à l'état d'acidalbumine ou d'autres dérivés des protéines, d'azote ammoniacal et d'azote aminé aliphatique.

On a calculé les diverses données des tableaux I à III en pour-cent de l'azote ingéré (tableau V) et en pour-cent de l'azote contenu dans la région de l'estomac examinée (tableau VI), celles des tableaux I à IV en pour-cent de l'azote incoagulable de la région du tube digestif envisagée (tableau VII).

TABLEAU IV.

Azote trouvé dans la première portion de l'intestin grêle à l'état incoagulable

Chien en expérience	Durée de la digestion, en heures	en grammes								en milligrammes	
		sous forme d'acid-albumine	sous forme de protéoses			sous forme d'autres produits			total		
			hétéroalbumose et protéalbumose	autres protéoses	total	précipités par l'acide phosphotungstique	non précipités par l'acide phosphotungstique	total		à l'état d'azote ammoniacal	à l'état d'azote aminé aliphatique
A	1	0·0000	0·0143	0·0191	0·0334	0·0672	0·0224	0·0896	0·1230	10·38	13·14
B	2	0·0000	0·0024	0·0022	0·0046	0·0140	0·0234	0·0374	0·0420	3·15	7·20
C	3	0·0000	0·0037	0·0042	0·0079	0·0745	0·1287	0·2032	0·2111	10·24	10·59
D	4	0·0000	0·0023	0·0035	0·0058	0·0651	0·0436	0·1087	0·1145	3·67	16·78
E	5	0·0006	0·0070	0·0051	0·0121	0·0610	0·1123	0·1733	0·1860	13·49	36·62
F	6	0·0000	0·0261	0·0282	0·0543	0·0569	0·0736	0·1305	0·1848	13·65	40·97
G	7	0·0000	0·0202	0·0416	0·0618	0·0405	0·0506	0·0911	0·1529	5·88	32·63
H	8	0·0000	0·0046	0·0048	0·0094	0·0243	0·0541	0·0784	0·0878	5·77	25·42
I	9	traces	0·0012	0·0087	0·0099	0·0293	0·0202	0·0495	0·0594	2·40	5·00
J	10	0·0000	0·0124	0·0327	0·0451	0·0736	0·0339	0·1075	0·1526	13·24	27·62

De plus, dans ce dernier tableau, on a établi la moyenne des résultats obtenus dans cette série d'expériences.

On n'a pas mentionné dans les tableaux I à IV les résultats donnés par la réaction du biuret dans les liquides privés des protéines, de l'acidalbumine et des protéoses, car elle n'a jamais fait défaut. Par conséquent, les deux régions de l'estomac et la première portion de l'intestin grêle contenaient toujours des peptones vraies de *Kühne*.

Comme dans toutes les séries d'expériences relatives à la digestion gastrique des protéines, on retrouve de moins en moins d'azote dans l'estomac au fur et à mesure qu'on s'éloigne du repas. Conformément aux résultats des

TABLEAU V.

Chien en expérience	Durée de la digestion, en heures	Portion de l'estomac examinée	Pour-cent de l'azote ingéré retrouvé dans l'estomac											Disparus de l'estomac
			à l'état coagulable	à l'état incoagulable							total			
				sous forme d'acid-albumine	sous forme de protéoses			sous forme d'autres produits						
					hétéroalbumose et protoalbumose	autres protéoses	total	précipités par l'acide phosphotungstique	non précipités par l'acide phosphotungstique	total		à l'état d'azote ammoniacal	à l'état d'azote aminé aliphatique	
A	1	corps	61·80	0·07	7·36	6·61	13·97	0·36	0·66	1·02	15·06	76·86	0·03	0·59	
		région prépylorique	3·56	0·01	0·62	0·66	1·28	0·22	0·52	0·74	2·03	5·59	0·03	0·11	
		estomac total	65·36	0·08	7·98	7·27	15·25	0·58	1·18	1·76	17·09	82·45	0·06	0·70	17·55
B	2	corps	56·62	0·03	5·70	6·27	11·97	0·55	1·32	1·87	13·87	70·49	0·35	0·98	
		région prépylorique	4·11	0·00	0·52	1·00	1·52	0·05	0·99	1·04	2·56	6·67	0·15	0·22	
		estomac total	60·73	0·03	6·22	7·27	13·49	0·60	2·31	2·91	16·43	77·16	0·50	1·20	22·84
C	3	corps	45·61	0·01	1·83	0·65	2·48	0·24	0·08	0·32	2·81	48·42	0·05	0·07	
		région prépylorique	1·48	traces	0·19	0·11	0·30	0·77	0·31	1·08	1·38	2·81	0·05	0·04	
		estomac total	47·09	0·01	2·02	0·76	2·78	1·01	0·39	1·40	4·19	51·23	0·10	0·11	48·77
D	4	corps	29·35	0·81	4·38	4·75	9·13	1·60	0·20	1·80	11·74	41·09	traces	0·54	
		région prépylorique	2·70	0·13	0·51	0·77	1·28	0·48	0·23	0·71	2·12	4·82	traces	0·10	
		estomac total	32·05	0·94	4·89	5·52	10·41	2·08	0·43	2·51	13·86	45·91	traces	0·64	54·09
E	5	corps	14·99	0·02	2·61	8·08	10·69	1·47	0·77	2·24	12·95	27·94	0·03	0·70	
		région prépylorique	2·53	0·01	0·07	0·42	0·49	0·40	0·74	1·14	1·64	4·17	0·07	0·06	
		estomac total	17·52	0·03	2·68	8·50	11·18	1·87	1·51	3·38	14·59	32·11	0·10	0·76	67·89
F	6	corps	4·13	2·65	3·91	3·96	7·87	0·68	1·09	1·77	12·29	16·42	0·20	0·20	
		région prépylorique	0·46	0·40	0·54	0·65	1·19	0·09	0·42	0·51	2·10	2·56	0·08	0·09	
		estomac total	4·59	3·05	4·45	4·61	9·06	0·77	1·51	2·28	14·39	18·98	0·28	0·29	81·02
G	7	corps	1·70	0·02	1·43	1·19	2·62	0·07	0·13	0·20	2·84	4·54	0·09	0·07	
		région prépylorique	1·37	0·01	0·19	0·41	0·60	0·08	0·27	0·35	0·96	2·33	0·04	0·09	
		estomac total	3·07	0·03	1·62	1·60	3·22	0·15	0·40	0·55	3·80	6·87	0·13	0·16	93·13
H	8	estomac total	1·08	0·37	1·31	2·38	3·69	0·27	0·65	0·92	4·98	6·06	0·20	0·06	93·94
I	9	estomac total	0·20	0·01	0·81	1·65	2·46	0·74	0·44	1·18	3·65	3·85	0·15	0·09	96·15
J	10	estomac total	0·45	0·09	0·74	0·88	1·62	0·20	0·32	0·52	2·23	2·68	0·06	0·03	97·32

TABLEAU VI.

Chien en expérience	Durée de la digestion, en heures	Portion de l'estomac examinée	Pour-cent de l'azote contenu dans la région de l'estomac examinée									
			en grammes								en milligrammes	
			à l'état coagulable	à l'état incoagulable								
				sous forme d'acid-albumine	sous forme de protéoses			sous forme d'autres produits				
					hétéroalbumose et protoalbumose	autres protéoses	total	précipités par l'acide phosphotungstique	non précipités par l'acide phosphotungstique	total	total	à l'état d'azote ammoniacal	à l'état d'azote aminé
A	1	corps	80·41	0·09	9·58	8·59	18·17	0·46	0·87	1·33	19·59	0·04	0·76
		région prépylorique	63·66	0·09	11·04	11·86	22·90	3·94	9·41	13·35	36·34	0·53	2·05
		estomac total	79·27	0·09	9·68	8·82	18·50	0·70	1·44	2·14	20·73	0·07	0·85
B	2	corps	80·33	0·04	8·08	8·90	16·98	0·79	1·86	2·65	19·67	0·49	1·39
		région prépylorique	61·65	0·00	7·75	14·95	22·70	0·80	14·85	15·65	38·35	2·26	3·30
		estomac total	78·71	0·04	8·05	9·42	17·47	0·79	2·99	3·78	21·29	0·64	1·56
C	3	corps	94·20	0·01	3·79	1·33	5·12	0·52	0·15	0·67	5·80	0·11	0·15
		région prépylorique	50·79	0·06	6·70	4·06	10·76	27·27	11·12	38·39	49·21	1·80	1·54
		estomac total	91·82	0·02	3·95	1·48	5·43	1·97	0·76	2·73	8·18	0·20	0·22
D	4	corps	71·42	1·97	10·65	11·57	22·22	3·89	0·50	4·39	28·58	0·01	1·31
		région prépylorique	56·09	2·61	10·59	16·03	26·62	9·90	4·78	14·68	43·91	0·06	2·18
		estomac total	69·82	2·04	10·64	12·04	22·68	4·52	0·95	5·47	30·19	0·01	1·40
E	5	corps	53·66	0·07	9·33	28·94	38·27	5·24	2·76	8·00	46·34	0·12	2·51
		région prépylorique	60·59	0·13	1·78	10·08	11·86	9·68	17·74	27·42	39·41	1·57	1·50
		estomac total	54·56	0·08	8·35	26·49	34·84	5·81	4·71	10·52	45·44	0·32	2·38
F	6	corps	25·12	16·13	23·81	24·13	47·94	4·12	6·69	10·81	74·88	1·27	1·21
		région prépylorique	17·86	15·59	21·14	25·38	46·52	3·43	16·60	20·03	82·14	3·24	3·67
		estomac total	24·14	16·05	23·45	24·30	47·75	4·04	8·02	12·06	75·86	1·54	1·56
G	7	corps	37·42	0·49	31·55	26·31	57·86	1·38	2·85	4·23	62·58	2·03	1·54
		région prépylorique	58·63	0·20	8·08	17·86	25·94	3·45	11·78	15·23	41·37	1·48	3·82
		estomac total	44·62	0·39	23·58	23·44	47·82	2·08	5·89	7·97	55·38	1·84	2·32
H	8	estomac total	17·85	6·17	21·61	39·15	60·76	4·54	10·68	15·22	82·15	3·32	1·06
I	9	estomac total	5·21	0·15	21·04	43·05	64·09	19·06	11·49	30·55	94·79	3·79	2·26
J	10	estomac total	16·80	3·11	27·65	32·93	60·58	7·65	11·86	19·51	83·20	2·12	1·04

TABLEAU VII.

Pour-cent de l'azote incoagulable contenu dans la région du tube digestif examinée

Chien en expérience	Durée de la digestion, en heures	Portion du tube digestif examinée	sous forme d'acidalbumine	sous forme de protéoses			sous forme d'autres produits				
				hétéroalbumose et protoalbumose	autres protéoses	total	précipités par l'acide phosphotungstique	non précipités par l'acide phosphotungstique	total	à l'état d'azote ammoniacal	à l'état d'azote aminé aliphatique
A	1	corps de l'estomac	0·46	48·90	43·86	92·76	2·36	4·42	6·78	0·18	3·91
		région prépylorique	0·26	30·38	32·63	63·01	10·84	25·89	36·73	1·47	5·63
		estomac total	0·44	46·68	42·54	89·22	3·36	6·98	10·34	0·34	4·11
		intestin grêle	0·00	11·62	15·53	27·15	54·64	18·21	72·85	8·44	10·68
B	2	corps de l'estomac	0·22	41·07	45·22	86·29	4·02	9·47	13·49	2·49	7·09
		région prépylorique	0·00	20·21	38·98	59·19	2·09	38·72	40·81	5·89	13·65
		estomac total	0·19	37·82	44·25	82·07	3·71	14·03	17·74	3·08	7·45
		intestin grêle	0·00	5·71	5·24	10·95	33·33	55·72	89·05	7·50	17·14
C	3	corps de l'estomac	0·26	65·39	22·99	88·38	8·71	2·65	11·36	1·91	2·44
		région prépylorique	0·13	13·62	8·25	21·87	55·40	22·60	78·00	3·67	3·13
		estomac total	0·22	48·29	18·12	66·41	24·14	9·23	33·37	2·49	2·67
		intestin grêle	0·00	1·75	1·99	3·74	35·29	60·97	96·26	4·85	5·02
D	4	corps de l'estomac	6·89	37·26	40·48	77·74	13·63	1·74	15·37	0·03	4·57
		région prépylorique	5·96	24·11	36·51	60·62	22·53	10·89	33·42	0·15	4·96
		estomac total	6·75	35·25	39·88	75·13	14·99	3·13	18·12	0·05	4·63
		intestin grêle	0·00	2·01	3·06	5·07	56·85	38·08	94·93	3·21	14·65
E	5	corps de l'estomac	0·15	20·13	62·46	82·59	11·30	5·96	17·26	0·27	5·41
		région prépylorique	0·32	4·52	25·58	30·10	24·54	45·04	79·58	4·08	3·82
		estomac total	0·17	18·37	58·30	76·67	12·80	10·36	23·16	0·70	5·23
		intestin grêle	0·32	3·76	2·74	6·50	32·80	60·38	93·18	7·25	19·69
F	6	corps de l'estomac	21·54	31·79	32·23	64·02	5·41	8·93	14·44	1·70	1·62
		région prépylorique	18·98	25·73	30·91	56·64	4·17	20·21	24·38	3·95	4·47
		estomac total	21·16	30·91	32·03	62·94	5·32	10·58	15·90	2·03	2·04
		intestin grêle	0·00	14·12	15·26	29·38	30·79	39·83	70·62	7·39	22·17
G	7	corps de l'estomac	0·78	50·41	42·04	92·45	2·21	4·56	6·77	3·25	2·47
		région prépylorique	0·48	19·52	43·18	62·70	8·33	28·49	36·82	3·57	9·01
		estomac total	0·70	42·57	42·34	84·91	3·76	10·63	14·39	3·33	4·19
		intestin grêle	0·00	13·21	27·21	40·42	26·49	33·09	59·58	3·85	21·35
H	8	estomac total	7·52	26·30	47·66	73·96	5·52	13·00	18·52	4·04	1·29
		intestin grêle	0·00	5·24	5·47	10·71	27·67	61·62	89·29	6·57	28·82
I	9	estomac total	0·15	22·20	45·42	67·62	20·11	12·12	32·23	4·00	2·39
		intestin grêle	0·00	8·13	21·42	29·55	22·22	48·23	70·45	8·68	18·10
J	10	estomac total	3·74	33·24	39·57	72·81	9·19	14·26	23·45	2·54	1·25
		intestin grêle	0·00	2·02	14·64	16·66	49·33	34·01	83·34	4·01	8·40
moyenne générale des résultats		corps de l'estomac	4·33	42·14	41·32	83·46	6·81	5·40	12·21	1·40	3·93
		région prépylorique	3·73	19·72	30·87	50·59	18·27	27·41	45·68	3·25	7·38
		estomac total	4·10	34·17	41·01	75·18	10·29	10·43	20·72	2·26	3·53
		intestin grêle	0·03	6·76	11·25	18·01	36·94	45·02	81·96	6·17	16·60

recherches antérieures, la digestion a progressé plus vite pendant les trois premières heures consécutives à l'ingestion de la viande cuite de bœuf qu'ensuite. Après la septième heure, l'évacuation de l'estomac n'a plus que progressé très lentement. L'achèvement de la digestion gastrique a demandé 10 à 11 heures au lieu d'un peu plus de 8 lorsqu'il s'est agi de la viande cuite de cheval.

Une fois de plus, la présente série d'expériences vérifie que la digestion gastrique de la viande cuite s'effectue plus vite, chez le chien, pendant les premières heures du processus digestif qu'ensuite, si l'on se base sur l'azote disparu de l'estomac, l'azote coagulable et l'azote incoagulable trouvés dans ce viscère.

L'azote coagulable tend à diminuer graduellement avec le laps de temps écoulé depuis le repas, tant dans le corps de l'estomac que dans l'ensemble de cet organe. Cette diminution est moins nette dans la portion prépylorique de l'estomac et ne s'observe guère qu'à partir de la troisième heure après l'ingestion de viande cuite de bœuf.

L'estomac ne renferme que très peu d'acidalbumine, sauf chez les chiens D, F et H. On en trouve une moindre proportion dans la portion prépylorique de cet organe que dans le corps de l'estomac. Toutefois, chez le chien E, la proportion d'azote incoagulable à l'état d'acidalbumine l'emporte dans la région prépylorique de l'estomac. Cette portion de cet organe ne renferme pas du tout d'acidalbumine chez le chien B et en contient à peine chez les chiens A, C, E et G. Sauf chez le chien E, il n'existe pas d'acidalbumine, ou tout au plus des traces (chien I) dans la première portion de l'intestin grêle.

La proportion d'azote incoagulable à l'état de protéoses diminue du corps de l'estomac à la portion prépylorique de cet organe et surtout de celle-ci à l'intestin grêle. Tel est toujours le cas pour les protéoses précipitées par demi-saturation au moyen du sulfate de zinc. Il en est de même pour les autres albumoses, sauf chez les chiens F et G, chez lesquels les deux régions renferment à peu près la même proportion de ces produits.

Au contraire la proportion d'azote incoagulable à l'état de peptones et de composés abiurétiques s'accroît du corps de l'estomac à la région prépylorique de cet organe et de celle-ci à l'intestin grêle. Il en est constamment ainsi pour les produits précipités par l'acide phosphotungstique, à l'exception des chiens B et F, chez lesquels la portion prépylorique de l'estomac présente une teneur en ces composés moins élevée que le corps de l'estomac, et du chien C, chez lequel la région prépylorique contient une proportion plus considérable de ces composés que les deux autres parties du tube digestif examinées.

La proportion d'azote incoagulable à l'état de composés non précipités par l'acide phosphotungstique augmente du corps de l'estomac à la région prépylorique de cet organe et de celle-ci à l'intestin grêle, sauf chez le chien A, chez lequel la teneur en ces composés est plus grande dans la région prépylorique de l'estomac que dans l'intestin.

Les protéoses non précipitées par demi-saturation au moyen du sulfate de zinc l'emportent sur les autres quatre fois sur sept dans le contenu du corps de l'estomac, six fois sur sept dans la portion prépylorique de l'estomac, sept fois sur dix dans l'estomac total, huit fois sur dix dans l'intestin. La quantité d'azote incoagulable à l'état d'hétéroalbumose et de protoalbumose est, en réalité, à peu près la même que celle sous forme des autres protéoses dans le contenu de l'estomac du chien G et de l'intestin des chiens B et H.

Les peptones et les composés abiurétiques non précipités par l'acide phosphotungstique prédominent sur les produits abiurétiques précipités par ce réactif, sauf dans le corps de l'estomac chez les chiens C, D et E, dans la portion prépylorique de cet organe chez les chiens C et D, dans l'estomac total chez les chiens C, D, E et I, dans l'intestin grêle chez les chiens A, D et J.

Le tableau VIII permet de comparer la répartition de l'azote non coagulable du contenu de l'estomac et de celui de la première portion de l'intestin grêle entre l'acidalbumine, les protéoses et les autres produits de scindage des protéines après l'ingestion de viande cuite de bœuf, selon qu'on emploie la méthode par filtration ou celle par centrifugation.

TABLEAU VIII.

Portion du tube digestif examinée	Méthode expérimentale employée	Pour-cent de l'azote non coagulable sous forme d'acidalbumine		de protéoses		d'autres produits	
		chiffres extrêmes	moyenne	chiffres extrêmes	moyenne	chiffres extrêmes	moyenne
estomac total	filtration		traces	78·94 à 90·94	88·39	9·06 à 21·06	11·61
	centrifugation	0·15 à 21·16	4·10	62·94 à 89·22	75·18	10·34 à 33·37	20·72
première portion de l'intestin grêle	filtration			9·86 à 29·00	17·16	71·00 à 90·14	82·84
	centrifugation	0·00 à 0·32	0·03	3·74 à 40·42	18·01	59·58 à 96·26	81·96

Les chiffres relatifs à la méthode par filtration mentionnés dans ce tableau proviennent des multiples expériences effectuées antérieurement de cette façon [Zunz, 1910,2]. Il n'est question dans ce tableau que de l'estomac total et de la première portion de l'intestin grêle, parce que dans les diverses

expériences effectuées jusqu'à présent en séparant le contenu du corps de l'estomac de celui de la région prépylorique de cet organe, on n'a jamais fait ingérer des chiens de la viande cuite de bœuf. En présence des avantages de la méthode par centrifugations successives, il n'y a pas lieu de se préoccuper de combler cette lacune.

Pour l'estomac considéré dans son ensemble, on constate moins de protéoses, davantage d'acidalbumine d'une part ainsi que de peptones et de composés abiurétiques d'autre part, au moyen de la méthode par centrifugation qu'au moyen de celle par filtration. Pour la première portion de l'intestin grêle, les chiffres moyens obtenus pour la répartition de l'azote incoagulable sont les mêmes, quel que soit le procédé employé: pas d'acidalbumine ou seulement des traces, moins du cinquième à l'état de protéoses, plus des $\frac{4}{5}$ à l'état de peptones et surtout de composés abiurétiques.

Les peptones et les corps abiurétiques ne dépassent guère le cinquième de l'azote incoagulable dans l'estomac total lorsqu'on procède par filtrations successives. Ces composés peuvent représenter jusqu'au tiers de cet azote lorsqu'on opère par centrifugations successives. Ces dérivés des protéines l'emportent toujours sur les autres produits de la désintégration des substances albuminoïdes dans la première portion de l'intestin grêle, quelle que soit la méthode expérimentale employée.

Occupons nous maintenant du problème que nous nous étions posés, à savoir si l'on observe, lors de la digestion de la viande cuite de bœuf, les mêmes différences entre les résultats des procédés par filtrations et par centrifugations successives que lors de la digestion de la viande cuite de cheval.

La proportion d'azote incoagulable à l'état d'acidalbumine est un peu plus élevée, tout en restant relativement faible, dans les deux régions de l'estomac aussi bien après l'ingestion de viande cuite de bœuf qu'après celle de viande cuite de cheval lorsqu'on utilise la centrifugation que lorsqu'on emploie la filtration. Dans les deux cas, l'intestin ne renferme pas ou guère d'acidalbumine.

Ce parallélisme des résultats ne s'observe plus pour les protéoses et les autres dérivés des protéines. Après l'ingestion de viande cuite de cheval, la proportion d'azote incoagulable à l'état de protéoses est à peu près la même par les deux procédés dans le corps de l'estomac et dans l'ensemble de cet organe. Elle est un peu plus considérable lorsqu'on utilise la méthode par centrifugation dans la région prépylorique de l'estomac et dans la première portion de l'intestin grêle. Par contre, la proportion d'azote incoagulable à

l'état de peptones et de composés abiurétiques (polypeptides pour l'estomac, en outre acides aminés pour l'intestin), précipités ou non par l'acide phosphotungstique est un peu inférieure dans le corps de l'estomac par la méthode de la centrifugation que par celle de la filtration. Ce phénomène s'accentue davantage dans la portion prépylorique de l'estomac et encore plus dans la première partie de l'intestin grêle.

L'influence de la méthode utilisée pour la séparation des diverses espèces de dérivés des protéines n'est donc en aucune façon la même pour l'estomac total selon que le chien ingère de la viande cuite de cheval ou de bœuf. Elle est nulle dans l'intestin après l'ingestion de viande cuite de bœuf. Il est impossible pour le moment de reconnaître à quoi sont dues les différences signalées ci-dessus entre les résultats obtenus par les méthodes de filtration et de centrifugation selon l'espèce de viande ingérée.

TABLEAU IX.

Portion du tube digestif examinée	Espèce de viande cuite ingérée	Pour-cent de l'azote non coagulable en moyenne sous forme		
		d'acidalbumine	de protéoses	d'autres produits
corps de l'estomac	bœuf	4·33	83·46	12·21
	cheval	2·84	76·71	20·45
portion prépylorique de l'estomac	bœuf	3·73	50·59	45·68
	cheval	1·43	67·40	31·17
estomac total	bœuf	4·10	75·18	20·72
	cheval	2·35	37·31	20·34
première portion de l'intestin grêle	bœuf	0·03	18·01	81·96
	cheval	0·00	30·75	69·25

Quoi qu'il en soit, il est certain que le procédé par centrifugations successives est à l'abri de plusieurs causes d'erreur inhérentes à la méthode par filtrations successives.

Que ressort il du tableau IX destiné à comparer la répartition moyenne de l'azote non coagulable des contenus des diverses régions du tube digestif entre l'acidalbumine, les protéoses et les autres dérivés des protéines, selon l'espèce de viande cuite ingérée?

Remarquons avant tout que les données du tableau IX, comme du reste aussi celles du tableau X, relatives à l'estomac considéré dans son ensemble et à la première partie de l'intestin grêle représentent les moyennes d'un plus grand nombre de résultats que les chiffres ayant trait au corps de l'estomac et à la portion prépylorique de cet organe.

Il y a partout davantage d'acidalbumine après ingestion de viande cuite

de bœuf qu'après celle de viande cuite de cheval. Il y a davantage de protéoses dans le corps de l'estomac et dans l'estomac total après ingestion de viande cuite de bœuf qu'après ingestion de viande cuite de cheval, moins au contraire dans la portion prépylorique de cet organe et dans l'intestin grêle. Quant aux autres dérivés des protéines (peptones et polypeptides pour l'estomac, acides aminés en outre pour l'intestin), on en observe une proportion moindre après ingestion de viande cuite de bœuf qu'après celle de viande cuite de cheval dans le corps de l'estomac, une proportion plus considérable dans la portion prépylorique de cet organe, dans l'ensemble de ce viscère et dans la première portion de l'intestin grêle.

La désintégration des protéines semble donc être poussée en moyenne un peu moins loin dans le corps de l'estomac après l'ingestion de viande cuite de bœuf qu'après celle de viande cuite de cheval, un peu plus loin dans la portion prépylorique de l'estomac et dans la première partie de l'intestin grêle.

Arrivons en à l'azote ammoniacal et à l'azote aminé aliphatique. Après l'ingestion de viande cuite de bœuf, ces deux espèces d'azote n'ont jamais fait défaut dans les trois régions du tube digestif examinées.

Les proportions d'azote ammoniacal et d'azote aminé aliphatique s'accroissent toujours du corps de l'estomac à la portion prépylorique de cet organe et de celle-ci à l'intestin. Toutefois, chez le chien G, l'accroissement de l'azote ammoniacal est insignifiant. En outre, chez le chien E, la teneur en azote aminé aliphatique de la portion prépylorique de l'estomac est inférieure à celle du corps de cet organe.

Le contenu intestinal présente toujours une teneur en azote aminé aliphatique supérieure à sa teneur en azote ammoniacal. Il en est aussi ainsi pour la portion prépylorique de l'estomac, à l'exception des chiens C et E. Tel est encore le cas pour le corps de l'estomac, sauf chez les chiens F et G, et pour le contenu total de l'estomac des chiens A, B, C, D, E et G. Chez le chien F, la teneur en azote ammoniacal et celle en azote aminé aliphatique sont les mêmes dans l'estomac considéré dans son ensemble. Chez les chiens H à J, sacrifiés huit à dix heures après le repas et dont l'estomac ne renferme plus qu'une faible quantité de bol alimentaire, l'azote ammoniacal l'emporte sur l'azote aminé dans ce viscère.

Le tableau X a pour but de comparer les teneurs en azote ammoniacal et en azote aminé aliphatique des diverses régions du tube digestif après ingestion de viande cuite, soit de bœuf, soit de cheval.

Si l'on se base sur l'azote incoagulable, la teneur du contenu des deux régions de l'estomac en azote ammoniacal est, en moyenne, moindre après

TABLEAU X.

Portion du tube digestif examinée	Espèce de viande cuite ingérée	Azote ammoniacal, en pour-cent				Azote aminé aliphatique, en pour-cent			
		de l'azote incoagulable		de l'azote total		de l'azote incoagulable		de l'azote total	
		chiffres extrêmes	moyenne	chiffres extrêmes	moyenne	chiffres extrêmes	moyenne	chiffres extrêmes	moyenne
corps de l'estomac	bœuf	0·03 à 3·25	1·40	0·01 à 2·03	0·58	1·25 à 7·09	3·93	0·15 à 2·51	1·27
	cheval	0·72 à 5·64	2·66	0·08 à 2·33	0·77	0·00 à 4·23	1·67	0·00 à 0·85	0·47
portion prépylorique de l'estomac	bœuf	0·15 à 5·89	3·25	0·06 à 3·24	1·55	3·13 à 13·65	7·38	1·50 à 3·82	2·57
	cheval	1·32 à 7·96	3·69	0·56 à 3·51	2·10	1·23 à 10·79	5·38	0·55 à 4·80	3·02
estomac total	bœuf	0·05 à 4·04	2·21	0·01 à 3·79	1·38	1·29 à 7·45	3·53	0·22 à 2·38	1·46
	cheval	0·98 à 5·82	2·65	0·17 à 2·41	0·84	0·42 à 5·25	2·13	0·05 à 1·20	0·62
première portion de l'intestin grêle	bœuf	3·21 à 8·68	6·17			5·02 à 28·82	16·60		
	cheval	1·51 à 8·33	4·45			5·86 à 16·80	10·71		

ingestion de viande cuite de bœuf qu'après celle de viande cuite de cheval. Elle est, par contre, plus élevée dans le premier cas que dans le second pour ce qui concerne le contenu de la première portion de l'intestin grêle. On observe les mêmes résultats si l'on prend pour point de départ l'azote total, sauf pour la teneur moyenne en azote ammoniacal de l'ensemble de l'estomac. Celle-ci l'emporte après ingestion de viande cuite de bœuf sur le chiffre observé après ingestion de viande cuite de cheval. Ceci est dû au fait que, chez les trois chiens H à J, sacrifiés huit à dix heures après avoir mangé de la viande de bœuf et dont on n'a pu examiner que l'ensemble de l'estomac par suite du peu de contenu encore présent dans ce viscère à ces moments, il existe une proportion relativement considérable d'azote ammoniacal pour une proportion relativement faible d'azote coagulable.

La teneur moyenne en azote aminé aliphatique est plus élevée, par rapport à l'azote incoagulable, après ingestion de viande cuite de bœuf qu'après celle de viande cuite de cheval dans toutes les régions du tube digestif examinées. Il en est encore ainsi par rapport à l'azote total pour le contenu soit du corps de l'estomac, soit de tout cet organe, mais par contre plus pour le contenu de la portion prépylorique de ce viscère.

Comme dans les expériences effectuées avec la viande cuite de cheval, on ne parvient pas, chez les chiens ayant ingéré de la viande cuite de bœuf, à déceler de relations spéciales bien nettes entre la teneur en azote ammoniacal

ou en azote aminé aliphatique des diverses régions du tube digestif et soit leur teneur en acidalbumine, en protéoses ou en autres dérivés de la désintégration des protéines, précipités ou non par l'acide phosphotungstique, soit le laps de temps écoulé depuis le repas. Néanmoins la teneur relativement considérable de la première portion de l'intestin grêle en azote ammoniacal et surtout en azote aminé aliphatique doit être rapprochée de la prédominance des peptones et des composés abiurétiques dans cette portion des voies digestives. Ceci est, d'ailleurs, aussi le cas après l'ingestion de viande cuite de cheval.

Résumé.

1. Après l'ingestion de viande cuite de bœuf, l'estomac renferme en moyenne, chez le chien, une plus forte proportion d'azote incoagulable à l'état de protéoses, une moindre proportion d'une part à l'état d'acidalbumine, d'autre part à l'état de peptones et de composés abiurétiques, lorsqu'on sépare les diverses espèces de dérivés des protéines par centrifugations successives que lorsqu'on utilise à cet effet l'ancienne méthode des filtrations successives. Au contraire les chiffres moyens obtenus pour la répartition de l'azote incoagulable du contenu de la première portion de l'intestin grêle sont les mêmes quelle que soit la méthode expérimentale employée: pas d'acidalbumine ou seulement des traces, moins du cinquième à l'état de protéoses, plus des $\frac{4}{5}$ à l'état de peptones et surtout de composés abiurétiques.

2. L'influence de la méthode utilisée pour la séparation des diverses espèces de dérivés des protéines n'est en aucune façon la même pour le contenu de l'estomac selon l'espèce de viande cuite ingérée. Elle est nulle pour le contenu intestinal après l'ingestion de viande cuite de bœuf.

3. On constate en moyenne davantage d'acidalbumine dans le contenu du corps de l'estomac, dans celui de la portion prépylorique de cet organe et dans celui de la première portion de l'intestin grêle après ingestion de viande cuite de bœuf qu'après celle de viande cuite de cheval. Il y a davantage de protéoses dans le corps de l'estomac et dans l'ensemble de cet organe après ingestion de viande cuite de bœuf qu'après celle de viande cuite de cheval, moins au contraire dans la portion prépylorique de l'estomac et dans l'intestin grêle. Quant aux autres dérivés des protéines (peptones et polypeptides pour l'estomac, acides aminés en outre pour l'intestin), on en observe une proportion moindre après ingestion de viande cuite de bœuf qu'après celle de viande cuite de cheval dans le corps de l'estomac, une proportion plus

considérable par contre dans la portion prépylorique et dans l'ensemble de cet organe ainsi que dans la première partie de l'intestin grêle.

4. Tant la teneur en peptones et en composés abiurétiques que les teneurs en azote ammoniacal et en azote aminé aliphatique s'accroissent d'ordinaire du contenu du corps de l'estomac à celui de la portion prépylorique de cet organe et de celui-ci au contenu de la première partie de l'intestin grêle.

5. Il n'y a pas de relation bien nette entre le laps de temps écoulé depuis l'ingestion de viande cuite de bœuf par le chien et soit la répartition de l'azote incoagulable entre les divers groupes de dérivés des protéines, soit la teneur en azote ammoniacal ou en azote aminé aliphatique des diverses portions du tube digestif examinées.

6. La teneur moyenne en azote ammoniacal est moindre après ingestion de viande cuite de bœuf qu'après celle de viande cuite de cheval dans le contenu des deux régions de l'estomac, plus élevée par contre dans le contenu de la première portion de l'intestin grêle.

7. La teneur moyenne en azote aminé aliphatique est plus considérable dans le corps de l'estomac, dans la région prépylorique de cet organe et dans la première partie de l'intestin grêle après ingestion de viande cuite de bœuf qu'après celle de viande cuite de cheval.

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IV. A NOTE ON THE HOPKINS-COLE REACTION FOR PROTEIN.

By H. G. D. BREIDAHL.

From the Biochemical Laboratory, Melbourne University.

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This work was undertaken at the suggestion of Dr Rothera, owing to the observation which had been made by those teaching in this laboratory and in the Physiology Laboratory, that this reaction usually failed with the acid supplied to the laboratories.

This, of course, was very disappointing to both students and teachers, as the reaction is generally thoroughly reliable, and moreover, very definite.

It was early observed that if old sulphuric acid were resorted to on encountering such failures, the reaction obtained was satisfactory.

Just as the work was commencing, Mottram's paper appeared in the *Biochemical Journal* [1914], and the work was then confined to seeking practical methods of improving the refractory acids. These invariably gave the brown or yellow-brown ring described by Mottram as being due to excess of oxidising agent.

Moreover, as no improvement was effected by the addition of traces of oxidising agents, such as $FeCl_3$, HNO_3, $NaNO_2$, it was very naturally suggested that excess of oxidising agent was already present. It was further thought that the old acids which gave the reaction might have lost some volatile impurity, such as oxides of nitrogen, and that these might be got rid of by mechanical means. However, attempts to obtain an improvement by heating the acid in a flat dish for an hour, or by bubbling air through the acid heated under low pressure for 86 hours, were unsuccessful.

The action of some reducing agents was then investigated, and, of those tried, the following were found to be the most useful.

1. *Magnesium.* The powdered metal used took about 24 hours to settle out sufficiently. The acid was then improved to some extent, the colour of

the ring being a very dark brown-purple. It was not further improved by prolonged standing in contact with the metal.

2. *Sodium bisulphite.* The use of this salt appears to be the most ready means of improving the acid, but is rather wasteful on account of the relatively large amount needed. If the salt and acid are well mixed, the reaction will be given as soon as the acid has cleared, which generally takes about five minutes. The colour of the ring is a purple-violet, and acid treated in this way retained the improvement after three months.

3. *Zinc.* (*a*) *In powdered form.* The powder used was extremely fine, and it was some weeks before the acid was clear enough to perform the test satisfactorily. The acid then seemed to err on the side of having too little oxidising agent, as the colour given was a blue-lilac, quite unlike the violet colour usually obtained. On running some weak $NaNO_2$ solution on to the ring, it developed into the characteristic violet-purple.

(*b*) *In the granulated form.* This provides the most satisfactory method of dealing with large quantities of acid for practical purposes. The acid should be well mixed to ensure thorough contact with the metal, and left for two or three days. The ring obtained is a deep violet-purple of great intensity, and shows no sign of browning.

The reagents used throughout were 2 % egg-white solution and "reduced oxalic," prepared according to the directions of Hopkins and Cole [1901].

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V. ON INACTIVE LIPASE AND THE NATURE OF ITS CO-ENZYME.

By NOBUYOSHI UMEDA.

From the Physiological Laboratory, King's College, London.

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Some years ago the observation was made in this laboratory by Rosenheim [1910] that active pancreatic lipase, as contained in glycerol extracts of pig's pancreas, can be separated into two inactive fractions by mere filtration through filter paper. The insoluble moiety on the filter paper contains the thermolabile inactive enzyme, which is restored to its original activity by the addition of the filtrate. The thermostable substance contained in the latter was designated as the co-enzyme according to the current nomenclature. Rosenheim also described a convenient method by which inactive lipase may be obtained as a dry powder free from soluble proteoclastic and amyloclastic enzymes, electrolytes, fats and lipoids.

From preliminary experiments it was concluded that the co-enzyme dialyses readily, and seems to be soluble in dilute but not in absolute alcohol, and insoluble in ether. Its activity is lost on incineration of the active dialysate. Of other substances tested bile salts and glycerophosphates were also found to possess activating powers.

These observations were fully confirmed by Pekelharing [1912], who made the additional interesting statement that the ash of the co-enzyme solution produced intense lipoclasis when added to the inactive lipase, if it had previously been dissolved in hydrochloric acid. He ascribed to calcium ions the main rôle in this reaction, which he explained by the removal of fatty acid as an insoluble calcium soap. Pekelharing failed, however, to adduce proofs that calcium salts are present in any amount in the co-enzyme solution; further, the effect due to calcium salts seems to account only for a relatively small amount of the total activating power of the co-enzyme solution.

Rona and Bien [1914], whilst partially confirming Rosenheim's results, were unable to demonstrate the presence of the co-enzyme. These observers,

however, employed a phosphate mixture as a medium, in order to regulate the hydrogen ion concentration during the reaction. As it will be shown in this communication that phosphates by themselves exert a powerful activating action on inactive lipase, it is not surprising that these authors failed to find the presence of a co-enzyme in their extracts.

The observations of J. Mellanby and Woolley [1914] refer to the lipase in pancreatic juice. It is obvious that in this case a separation of enzyme and co-enzyme by mere filtration is impossible as the enzyme is in a soluble condition as distinguished from the suspended insoluble state in which it occurs in pancreatic extracts. Mellanby and Woolley made an unsuccessful attempt to effect a separation by alcohol. The impossibility of separating the lipase of pancreatic juice into enzyme and co-enzyme by means of alcohol precipitation does not, however, necessarily imply that their separation is impossible by other means.

The present investigation was undertaken in order to study in the first instance the activating power of the inorganic constituents of the co-enzyme solution. The action of electrolytes on pancreatic lipase has already been the subject of many investigations. The results have, however, been rather contradictory. Thus, to mention only two recent investigations, Terroine [1910] working with pancreatic juice and olive oil found an accelerating influence of Na, K, Mg and Ba salts, whilst Ca salts had no action. On the other hand, Mellanby and Woolley [1914] deny the influence of electrolytes altogether. Quite apart from the influence of the varying degree of emulsification of the zymolytes employed (fats or esters) it is obvious that both pancreatic juice and glycerol extracts of pancreas contain electrolytes in varying amount. Terroine has indeed attempted to remove the electrolytes by dialysis, but found that the lipase of pancreatic juice was destroyed in this process, probably by the action of the proteoclastic enzyme. Whilst therefore pancreatic juice and extracts appear to be unsuitable for the study of the action of electrolytes on their enzymes, this objection does not hold for the inactive lipase preparations made by Rosenheim's method, which can be obtained free from electrolytes and soluble enzymes by careful washing with water.

Whilst I was able to show conclusively that the inorganic constituents, especially the phosphates, contribute largely to the activity of the co-enzyme solution, the fact was incidentally established that the pancreatic co-enzyme is of a complex nature and that organic substances also are concerned in the reaction.

Experimental.

Preparation of inactive lipase.

2 kilos. of fresh pig's pancreas, freed as far as possible from visible fat, were minced and mixed with 4 litres of glycerol. The mixture was frequently stirred and usually allowed to stand for 24 hours. By straining through two layers of fine muslin, a turbid extract was obtained, which possessed considerable fat-splitting activity.

Portions of one litre of this extract were diluted with ten litres of distilled water and the milky fluid allowed to settle in tall cylinders in the ice box. As a rule a perfect separation is obtained at this stage, a white precipitate settling after 24 hours' standing from which the clear supernatant fluid can be easily siphoned off. The precipitate is again suspended in 10 litres of distilled water. Probably owing to the removal of the electrolytes the lipase remains now usually in a colloidal suspension, but by means of a centrifuge a separation can be easily effected.

Pekelharing recommends the addition of acetic acid until the fluid is distinctly acid to sensitive litmus paper. A very satisfactory separation is certainly produced by this modification, but there is considerable danger that trypsin and trypsinogen also are precipitated by the addition of acetic acid. I have always found that although a better yield and a better separation are obtained with the help of acetic acid, the resulting preparation is always less active lipoclastically and also possesses a marked proteoclastic activity when tested with congo-fibrin. The use of acetic acid is therefore not to be recommended when a highly active preparation is desired.

Three washings are usually sufficient to free the preparation from the co-enzyme. The flocculent precipitate is next suspended in an equal volume of alcohol and filtered through a Pukall's [1893] porous clay filter. The precipitate is washed on the filter with acetone and finally with ether. After this treatment it easily peels off in flakes from the filter and after drying in vacuo is obtained as a fine greyish-white powder.

Besides my own preparations I have used an inactive lipase which had been prepared over two years ago by the late Mr Myers-Ward and which was kindly put at my disposal by Dr Rosenheim. This preparation had not been so fully freed from co-enzyme as my own, but its activity after the addition of co-enzyme was somewhat higher than that of mine. It is interesting to note that this powder retained its potential energy although kept for two years under the ordinary conditions of the laboratory.

Preparation of co-enzyme solutions.

The fluid decanted from the precipitate obtained on diluting pancreatic glycerol extract with water, when boiled, filtered and concentrated contains the co-enzyme of lipase free from proteoclastic and other enzymes. The large percentage of glycerol, however, was thought to be in the way for the purpose of my experiments and I therefore used watery extracts of pancreas prepared according to Rosenheim's directions. 500 g. of minced pig's pancreas were boiled up with 500 cc. of distilled water, the mixture kept hot for 30 minutes and filtered after cooling. 620 cc. of extract were obtained which were preserved under toluene. On keeping, a sediment gradually formed mainly consisting of tyrosine; as this sediment was found to be devoid of any activating power, the clear supernatant fluid was used for the experiments.

The acidity of 1 cc. of the different preparations of co-enzyme solution varied from 1·4–2·5 cc. N/10 KOH. This amount, which remained constant after incubation with olive oil emulsion, was always allowed for.

Estimation of lipoclastic activity.

An emulsion of olive oil was used as the zymolyte and the degree of lipoclasis was determined by incubating the mixture of the enzyme and zymolyte for a given time (usually 17–20 hours) and titrating the fatty acids liberated with N/10 KOH. Alcohol was added in sufficient amount to keep the percentage of the titrated solution at least at 50 % alcohol and phenolphthalein was used as an indicator.

In order to obtain concordant results it is important that certain precautions are observed in carrying out these experiments. A short description of the routine followed in this laboratory may therefore not be out of place.

(*a*) Preparation of the oil emulsion. The quality of the olive oil employed is of considerable importance. On testing the activity of a pancreatic extract on a series of various commercial olive oils, it was found that under identical conditions some oils were easily attacked whilst others were hardly hydrolysed at all. One of these resisting oils was further investigated and was found to contain about 10 % of Arachis oil, an oil which is very resistant to the action of pancreatic lipase. The oil used in this laboratory is described as "Huile d'olive vierge" J. L. Duret & Co.

Before preparing the emulsion, the amount of N/10 KOH necessary to neutralise the free acid present is estimated by titrating 5 cc. of olive oil[1] without the addition of alcohol, until a slight permanent pink reaction to phenolphthalein is reached. Not more than 0·5 cc. N/10 KOH for 5 cc. of olive oil should be required. The calculated amount having been added to 50 cc. of

[1] For the exact measurement of small quantities of oil, emulsion or glycerol extracts, glass syringes are used made by grinding a glass rod into 5 cc. pipettes of about 25 cm. length, graduated into 1/10 cc.

oil (about 5 cc. N/10 KOH) a uniform emulsion is obtained by means of a glass syringe, the oil being alternately drawn up and discharged in a shallow glass dish. 2·5 cc. or 5·0 cc. of this emulsion, which should not be made too thick, were used for each experiment.

(*b*) 10 mg. of inactive lipase is sufficient to obtain a well-marked reaction. It was used in a 0·2 % or 1 % suspension in water, which is prepared by first grinding the dry inactive lipase in a small mortar with a few drops of water into a fine paste and gradually adding the necessary volume of water. The suspension was freshly prepared for each set of experiments and care must be taken not to allow it to settle before measuring off the necessary amounts.

(*c*) The order in which enzyme, co-enzyme and zymolyte are mixed is of importance. In my experiments the enzyme suspension was measured into a series of small flasks first, the co-enzyme (or solution to be tested) being added next. 2·5 or 5 cc. of oil emulsion were mixed with these substances by shaking each flask for exactly the same time (20 seconds), it having been found that irregular results are obtained, unless this precaution is observed. The addition of toluene during digestion is unnecessary, as the large percentage of oil prevents bacterial development.

In the following table are given the results of experiments, made at various times, with the same preparations of inactive lipase and co-enzyme. In the last column is given the activity of the mixture of inactive enzyme and co-enzyme expressed in cc. N/10 KOH, the acidity due to co-enzyme solution and that produced by the inactive lipase alone having been deducted.

	Inactive lipase (10 mg. in 1 cc. water) cc.	Co-enzyme cc.	Time of incubation, hours	N/10 KOH cc.	Activity
No. 1	1	–	17	0·5	7·1
	1	1	,,	7·6	
	1	–	19	0·9	7·6
	1	1	,,	8·5	
	1	–	,,	0·9	7·0
	1	1	,,	7·9	
No. 2	1	–	17	2·5	10·5
	1	1	,,	13·0	
	1	–	16	2·5	8·9
	1	1	,,	11·4	
	1	2	,,	13·1	10·6
	1	–	17	2·5	9·4
	1	1	,,	11·9	

In the preparation of No. 1, acetic acid had been used and the co-enzyme had been carefully removed. The preparation had a very low initial activity, but its activity after the addition of the co-enzyme solution was not as great as that of No. 2, which had been prepared by Mr Myers-Ward without the use of acetic acid. These results fully confirm Rosenheim's observations and prove that the lipase of glycerol extracts of pig's pancreas can be separated into an inactive lipase and a co-enzyme.

The action of the constituents of the ash of the co-enzyme.

After incineration of the active dialysate of a co-enzyme solution, Rosenheim found the filtered ash solution devoid of any action on the inactive lipase. This result may have been due to the alkalinity of this solution. Pekelharing observed that the ash of the co-enzyme solution (not dialysed) if neutralised with hydrochloric acid, conferred a strong lipoclastic activity on the inactive lipase. No quantitative experiments, however, are described by Pekelharing. For the purpose of my experiments I carefully incinerated portions of 5 cc. of watery pancreas extracts in a platinum dish and dissolved the ash in a small volume of water, after adding a drop of methyl red and the necessary amount (0·2–1·0 cc.) of N/10 HCl. The solution was evaporated to dryness in order to remove any excess of hydrochloric acid and the residue was dissolved again in water. The clear solution was made up to 5 cc. and its action compared with that of the original co-enzyme solution.

5 cc. of a 0·2 % suspension of inactive lipase and 5 cc. of oil emulsion were used. Time of incubation: 17 hours. The necessary corrections (in cc. N/10 KOH) were: 1 cc. co-enzyme (*a*) 2·0; 1 cc. ash solution (*a*) 0·3; 1 cc. co-enzyme (*b*) 1·6; 1 cc. ash solution (*b*) 0·4. The acidity due to inactive lipase alone was 2·5 cc. N/10 KOH. The results are given in the following table.

		Degree of lipoclasis	
		Titrated value N/10 KOH	Corrected value N/10 KOH
1.	Co-enzyme (*a*) cc.	cc.	cc.
	1	12·2	7·7
	1	12·4	7·9
	Ash solution (*a*) cc.		
	1	10·3	7·5
	1	9·2	6·4
2.	Co-enzyme (*b*) cc.		
	1	13·3	9·2
	1	13·0	8·9
	Ash solution (*b*) cc.		
	1	11·2	8·3
	1	10·9	8·0

From these results it seems that the solution of the ash of the co-enzyme, when dissolved in hydrochloric acid, possesses an activating power nearly equal to that of the original co-enzyme.

Dialysis experiments.

Two different preparations of the co-enzyme solution were dialysed, one through a parchment diffusion shell (Schleicher and Schüll) and one through a sheep's appendix according to W. Wiechowski [1907]. The dialysis against distilled water was continued for four days, until a complete absence of chlorides in the dialysate was observed. The dialysate and the contents of the dialysing membranes were concentrated to the original volume. 5 cc. of each of these solutions were incinerated as above described and the activity of the ash-solutions on inactive lipase compared with that of the original solutions. 5 cc. of olive oil emulsion and 10 mg. of inactive lipase in 5 cc. of water were used in each case. Time of incubation: 18 hours. The necessary corrections were made as before.

The results are given in the following table.

		Degree of lipoclasis	
	Dialysate cc.	Titrated value cc. N/10 KOH	Corrected value cc. N/10 KOH
1	1	10·9	7·6
	2	12·3	8·4
2	1	10·5	7·4
	2	14·7	9·7
	Ash solution of dialysate cc.		
1	1	9·8	6·8
	2	9·6	7·3
2	1	9·3	6·8
	2	11·8	9·0
	Dialysed solution cc.		
1	1	3·0	1·0
	2	3·7	1·4
2	1	2·5	0·7
	2	3·5	1·5
	Ash solution of dialysed solution cc.		
1	1	2·6	0·8
	2	3·2	1·3
2	1	2·0	0·2
	2	2·8	0·9

These results prove that nearly the whole of the activating substances passes through the dialysing membranes as stated by Rosenheim. The ash of the incinerated dialysate, if dissolved in hydrochloric acid, produces practically the same effect as the original dialysate.

From these experiments it would appear that the activity of the co-enzyme solution is not lost by incineration. This conclusion is, however, not valid in its entirety as will be seen from the following experiments. It has been found in the course of some unpublished work carried out by the late Mr Myers-Ward in this laboratory that the co-enzyme solution could be separated into two fractions by means of absolute alcohol. Both of these fractions, the alcohol-soluble as well as the alcohol-insoluble fraction, were able to activate inactive lipase. It seemed therefore of interest, to investigate the action of the ash constituents of these fractions.

Fractionation experiments with alcohol.

10 cc. of watery extract of pancreas were poured into 90 cc. of boiling absolute alcohol. A flocculent precipitate formed, from which the alcoholic solution was decanted. On the addition of more boiling alcohol the precipitate became granular and was easily filtered off, washed with alcohol and ether, and dried in vacuo.

The alcoholic solution was evaporated to dryness and the residue again

	Degree of lipoclasis	
	Titrated value cc. N/10 KOH	Corrected value cc. N/10 KOH
Alcohol-soluble fraction of co-enzyme cc.		
1	6·5	3·3
1	6·0	2·8
Ash solution cc.		
1	3·2	0·6
1	3·0	0·4
Alcohol-insoluble fraction of co-enzyme cc.		
1	8·5	5·0
1	8·3	4·8
Ash solution cc.		
1	6·0	3·2
1	5·8	3·0
Original co-enzyme cc.		
1	13·0	9·1
Ash solution cc.		
1	9·0	6·2

taken up with boiling alcohol. The insoluble part was added to the main insoluble precipitate.

Before use the alcohol was evaporated and the residue dissolved in 10 cc. water. The alcohol-insoluble fraction also was dissolved in 10 cc. water.

5 cc. of each of these solutions were incinerated as described above and the action of these several fractions on inactive lipase compared with that of the original co-enzyme solution.

10 mg. of inactive lipase, 5 cc. oil emulsion. Time of incubation: 19 hours. The following corrections (cc. N/10 KOH) were necessary for the various solutions: 1 cc. alcohol-soluble fraction 0·7; 1 cc. ash solution 0·1; 1 cc. alcohol-insoluble fraction 1·0; 1 cc. ash solution 0·3; 1 cc. original watery extract 1·4; 1 cc. ash solution 0·3; 10 mg. inactive lipase 2·5. The results are given in the table on p. 45.

It is evident from these results that both the alcohol-soluble and the alcohol-insoluble fraction of the co-enzyme are able to activate inactive lipase, the former, however, to a smaller degree. Moreover, the greatest part of its activating power is destroyed by incineration. It is clear, therefore, that organic substances as well as electrolytes are concerned in this reaction.

I had also at my disposal an alcoholic extract of a co-enzyme solution prepared by the late Mr Myers-Ward. This had been prepared two years ago from a large quantity of material and had been purified to such an extent that its residue was completely alcohol-soluble. The solution had a slight deposit which was filtered off. When tested on the same inactive lipase preparation as previously, it was found to have lost some of its activity. This may have been contained in the insoluble deposit which was unfortunately no longer available. For comparison the results obtained by Mr Myers-Ward are given together with my own in the following table. The necessary corrections have been made.

	Activity	
Alcohol-soluble fraction of co-enzyme, cc.	(1912)	(1914)
1	12·7	5·1
1	12·7	5·1
Ash solution of same, cc.		
1	2·2	1·1
1	2·5	1·4

As was to be expected, the percentage of ash of this alcohol-soluble fraction was very small, namely 0·09 %, and it seemed to consist to a large extent of phosphoric acid. This led me to the investigation of the action of phosphoric

acid and phosphates on inactive lipase. Except for Rosenheim's statement [1910] that glycerophosphates activate inactive lipase, no attention seems to have been given up till now to the influence of phosphates on fat splitting. In this connection it is interesting to note that according to Plimmer and Scott [1908] the pancreas alone of all the tissues examined contains phosphoproteins, and that these substances disappear from the pancreas during the secretion of pancreatic juice and are transformed to the largest extent into water-soluble phosphates which appear in the pancreatic juice [Plimmer and Kaya, 1910].

Before proceeding to carry out experiments with phosphates on inactive lipase, I estimated quantitatively the percentage of inorganic and organic phosphorus compounds in the co-enzyme solution employed.

Total P was estimated in 5 cc. of the solution by Neumann's method. For the estimation of organic P, 15 cc. of the solution were precipitated with the same volume of magnesia mixture and P estimated in the filtrate. Inorganic P was estimated by difference. The following results were obtained: Total P: 0·36 %; organic P: 0·25 %; inorganic P: 0·11 % The total ash of the co-enzyme solution was 0·59 %.

Hence the percentages of P in the ash are:

Total P	61 %.
Organic P	42 %.
Inorganic P	19 %.

It will be seen that the ash contains a very large percentage of P, 42 % of which is present in the original solution as organic phosphorus compounds (nucleic acid, organic phosphates) and 19 % as inorganic phosphates.

The action of phosphates on inactive lipase.

The action of very dilute solutions of phosphoric acid was first examined. Increasing quantities of these were added to 5 cc. of a 0·2 % suspension of inactive lipase. After bringing up to equal volume with water and adding 5 cc. of oil emulsion the mixtures were incubated for 17 hours. The acidity due to the added phosphoric acid was allowed for. The results are given in the following table.

H_3PO_4 added cc.	Molecular concentration of H_3PO_4 in mixture	Degree of lipoclasis: Titrated value cc. N/10 KOH	Degree of lipoclasis: Corrected value cc. N/10 KOH
0·1 M/100	M/17000	2·6	0
0·5 „	M/1400	3·1	+0·4
1·0 „	M/700	9·3	+6·5
0·2 M/10	M/350	11·7	+8·6
0·5 „	M/140	1·6	−2·4
Inactive lipase		2·5	0
Inactive lipase + co-enzyme (1 cc.)		13·5	9·4

These experiments show conclusively that very dilute phosphoric acid solutions are able to activate inactive lipase to a marked degree, whilst an inhibition is exerted if a certain limit is overstepped. I have not investigated how far this result is connected with the optimum of hydrogen ion concentration for pancreatic lipase, but in the following experiment mixtures of primary and secondary phosphates were employed which enabled me to judge roughly the hydrogen ion concentration of the digestion mixture.

A M/15 solution of primary potassium phosphate and a M/15 solution of secondary sodium phosphate were prepared according to Sörensen [1912]. 6 cc. of definite mixtures of these were added to the usual mixture of inactive lipase and oil emulsion. The mixtures were incubated for 19 hours. In the following table are given the composition of the various phosphate mixtures, their titration values against phenolphthalein and their hydrogen ion concentration together with the effect they produced on inactive lipase.

	Phosphate mixture		Titration value of 6 cc. in cc. N/10 KOH	P_H	Degree of lipoclasis	
	Primary cc.	Secondary cc.			Titrated value cc. N/10 KOH	Corrected value cc. N/10 KOH
1.	10·0	0·0	3·6	(4·494)	6·3	1·8
2.	9·75	0·25	2·4	5·288	6·2	2·9
3.	9·0	1·0	1·7	5·906	7·1	4·5
4.	7·0	3·0	1·3	6·468	6·6	4·4
5.	5·0	5·0	0·8	6·813	8·6	9·6
6.	3·0	7·0	0·5	7·168	11·2	9·8
7.	1·0	9·0	0·2	7·731	11·3	10·2
8.	0·0	10·0	0·1 N/10 H_2SO_4	(9·182)	6·7	5·9
	Inactive lipase				0·9	—
	Inactive lipase + co-enzyme				8·5	7·6

It is evident that all these phosphate mixtures appear to activate lipase. The action is most marked when a mixture in the proportion of 3 : 7 or 1 : 9 of primary and secondary phosphates is used. This fact incidentally explains also the failure of Rona and Bien [1914] to detect the presence of the co-enzyme in their extracts, as its effect was hidden by that produced by the phosphate mixtures employed by them in order to regulate the hydrogen ion concentration of their mixtures.

How far the action of phosphates is due to a direct action on the enzyme itself or to the production of a favourable reaction (hydrogen ion concentration) for its action, must be left for future investigations.

The action of calcium salts on inactive lipase.

The fact that Pekelharing [1912] ascribes to calcium the greatest part of the activating effect of the ash solution of the co-enzyme, without

adducing, however, any figures to show that calcium is present in sufficient amount in this solution, induced me to estimate quantitatively the calcium contents of my extracts. Qualitative experiments showed that its quantity was very small and 200 cc. of the extract were therefore taken for the estimation, which was made by Aron's method, as modified by R. von der Heide [1914]. 200 cc. gave 0·0436 g. calcium oxalate = 0·02 % $CaCl_2$.

A series of experiments was next carried out, in which increasing amounts of calcium chloride were added to 5 cc. of a 0·2 % suspension of inactive lipase. After bringing up to equal volume with water and adding 5 cc. of olive oil emulsion the mixtures were incubated for 17 hours. The action of calcium chloride was compared with that of the original co-enzyme and its ash solution. The usual controls were made.

Calcium chloride content of mixture	Degree of lipoclasis: Titrated value cc. N/10 KOH	Degree of lipoclasis: Corrected value cc. N/10 KOH
M/7000	2·5	0
M/1400	2·5	0
M/700	5·0	+2·5
M/70	4·5	+2·0
M/7	3·1	+0·6
M/3·5	2·0	−0·5
Inactive lipase alone	2·5	0
Inactive lipase + co-enzyme (1 cc.)	12·4	8·3
Inactive lipase + ash solution (1 cc.)	11·0	8·1

From these results we may conclude that calcium chloride, if present in the digestion mixture to the amount of M/7000 to M/1400 has no effect on the lipoclasis. As the calcium content of 1 cc. of the ash solution of the co-enzyme corresponds to a M/4116 solution, it seems justifiable to conclude that the action of the ash solution is not due to calcium. Even if it were present in much larger proportion (M/700 to M/70) its effect would still be far below that of the ash solution itself.

I have also examined in a similar way the action of primary calcium phosphate, $CaH_4(PO_4)_2$, as this salt might possibly be present in the solution of the ash. The following results were obtained.

$CaH_4(PO_4)_2$ solution added cc.	Titration value cc. N/10 KOH	$CaH_4(PO_4)_2$ contents of the mixture	Degree of lipoclasis: Titrated value cc. N/10 KOH	Degree of lipoclasis: Corrected value cc. N/10 KOH
0·1 M/100	trace	M/7000	2·8	0·6
1·0 ,,	0·4	M/700	4·0	1·4
2·0 ,,	0·8	M/350	7·6	4·6
1·0 M/20	1·3	M/140	7·1	3·6
1·5 ,,	1·9	M/95	5·7	1·6
2·0 ,,	2·5	M/70	4·9	0·2

These experiments show that primary calcium phosphate has a certain amount of activating action, which must, however, be ascribed to its phosphoric acid ions if we consider the results of the preceding experiments. But even its maximum effect (M/350) accounts for only about 50 % of that of the ash solution of the co-enzyme.

Whilst therefore admitting the possibility that calcium salts may act in the way suggested by Pekelharing[1] (*i.e.* by precipitating a small amount of fatty acid as an insoluble soap), it seems from the above experiments that its rôle as an activator of inactive lipase is in any case an insignificant one.

The action of sodium chloride on inactive lipase.

As sodium chloride forms a constituent of the co-enzyme solution, I have made a few experiments with this salt. 5 cc. of a 0·2 % suspension of inactive lipase to which increasing amounts of sodium chloride solution had been added, were brought up to equal volume with water, mixed with 5 cc. of oil emulsion and incubated for 19 hours. The results are given in the following table.

NaCl concentration of the mixture	Degree of lipoclasis	
	Titrated value cc. N/10 KOH	Corrected value cc. N/10 KOH
M/700	1·8	0·9
M/175	2·2	1·3
M/100	3·0	2·1
M/70	3·9	3·0
M/17·5	2·8	1·9
M/10	2·7	1·8
M/7	2·2	1·3
Inactive lipase alone	0·9	0
Inactive lipase + co-enzyme (1 c.)	9·5	7·0

It would seem from these experiments that the action of sodium chloride is not of any great significance. Its effect increases to a certain limit, after which it decreases again. It may be mentioned here, however, that Dr Shaw-Mackenzie and the late Mr Myers-Ward occasionally observed a considerable activating influence of sodium chloride on inactive lipase. No explanation has as yet been found for these contradictory results.

Discussion.

In considering the state of our present knowledge of the conditions governing the activities of pancreatic lipase, it is essential to differentiate sharply between the lipase of glycerol extracts of pancreas and the lipase as

[1] See, however, Rona and Bien [1914], whose observations on the action of calcium on pancreatic and serum lipase do not favour this explanation.

contained in pancreatic juice. The activity of the latter is in no way impaired by ordinary filtration and it is evident that the enzyme exists in a soluble condition in pancreatic juice. In glycerol extracts of pancreas, however, the material to which its lipoclastic activity is due occurs in a colloidal suspended condition and it requires a soluble co-enzyme for its activation.

The changes by means of which the insoluble lipase of the organ is transformed into the soluble enzyme of the pancreatic juice during the process of the secretory activity of the organ, are at present still unknown. With the methods at our disposal at present, it has also been found impossible to demonstrate the presence of a co-enzyme for the soluble lipase of pancreatic juice. It is, however, to be expected that the study of substances which possess a solvent action on the inactive lipase of glycerol extracts may give us a clue as to the conditions which govern the solubility of the lipase of pancreatic juice.

Various substances are now known which are able either to activate inactive lipase or to accelerate the action of active lipase. It seems that these substances may be divided into three groups: (1) The salts of Na, K, Ca, Ba, Mg; (2) organic and inorganic phosphates; and (3) bile salts and their derivatives. Very little is known, however, with regard to their mode of action.

In the case of the inactive lipase of pancreatic extracts any explanation is complicated by the fact that we are dealing with an insoluble enzyme acting on an equally insoluble zymolyte (fat). The action of neutral salts may therefore be partly due to a physical effect either on the degree of emulsification of the fat or on the degree of colloidal suspension of the enzyme. Pekelharing has further made it probable that the relatively small effect of electrolytes may be ascribed to the removal of one of the products of reaction (fatty acid) from the system.

This explanation seems, however, hardly admissible for the substances belonging to the second group. Their action may be due to a direct action on the enzyme or perhaps in the case of phosphates to the fact that their hydrogen ion concentration corresponds to the optimum activity of the enzyme. Another explanation of the action of phosphates may be their possible rôle in linking the inactive enzyme to the fat and giving rise to the intermediate formation of glycerophosphoric acid.

In the last class of substances, the bile salts, it has been made most probable by Rosenheim's observations that their active constituent, cholalic acid, acts directly on the enzyme. A similar conclusion has also been reached by Terroine [1910].

The nature of the activating substances contained in a boiled watery extract of pancreas is not yet completely cleared up. Bile salts are absent and of the inorganic substances it seems that the main rôle is due to phosphates. The nature of the organic activator remains still to be discovered.

The expenses of this research have been defrayed out of a grant made to Dr Rosenheim from the Government Grant Committee of the Royal Society.

Conclusions.

(1) Phosphates represent the most active inorganic constituents of the co-enzyme of inactive lipase obtained from pancreatic extracts.

(2) Organic substances, probably organic phosphates, are also concerned in the activation of inactive lipase.

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VI. ON THE AUXO-LIPASE OF SERUM.

By KWANJI TSUJI.

From the Physiological Laboratory, King's College, London.

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The addition of small amounts of serum to pancreatic extracts increases their fat-splitting power to a remarkable degree. This fact was established by Pottevin [1903] and independently by J. A. Shaw-Mackenzie in his joint work with O. Rosenheim on the action of lipase contained in glycerol extracts of pancreas on olive oil and ethyl butyrate [1910]. The lipase of pancreatic juice (dog) as well as that of human pancreatic juice [Shaw-Mackenzie, 1912] are similarly influenced by serum. Minami [1912], apparently without knowledge of the previous work, recorded similar observations, using monobutyrin as the zymolyte.

With regard to the chemical nature of this accelerating substance, for which I propose the name of auxo-lipase[1], Rosenheim and Shaw-Mackenzie only made some preliminary statements, according to which it seemed to withstand the temperature of boiling water and was not destroyed by putrefaction. Further, it appeared to be soluble in dilute alcohol and to dialyse through membranes[2].

In a recent paper J. Mellanby and Woolley [1914] made a useful suggestion with regard to the nature of this reaction, which lends itself to experimental investigation. They consider that the increase of lipoclastic power on the addition of serum to pancreatic extracts is due to the protection of the lipase from the action of trypsin by the antitrypsin contained in the added serum.

The properties of auxo-lipase, as far as they have been investigated, suggest a connection with the co-enzyme of pancreatic lipase. The purpose of this investigation was in the first instance to compare the behaviour of

[1] Similar auxo-substances in serum have already been described in literature (see M. Falk, [1914]; Guggenheimer, Minami).

[2] I am informed that in later experiments Rosenheim and Shaw-Mackenzie were unable to confirm this preliminary statement.

these two substances and to establish their identity or difference. Further, the relationship of auxo-lipase to the antitrypsin of serum was experimentally investigated. This relationship is of interest since it has been shown by Shaw-Mackenzie [1912, 1913] that variations in amount of the auxo-lipase of serum together with variations in amount of antitrypsin may be of diagnostic value in certain pathological conditions.

Experimental.

Since the action of the auxo-lipase is not specific, I have limited my work, for convenience sake, to the investigation of ox-serum. Olive oil was chosen as the zymolyte, as this fat gives much larger values than those obtained by the action of pancreatic lipase on various esters of the lower fatty acids. The method used for the estimation of the lipoclastic activity was the same as that of Rosenheim and Shaw-Mackenzie. The precautions necessary for comparable results have been fully described in the communication of Umeda [1915]. Instead of phenolphthalein, I used, however, a 2 % alcoholic solution of thymolphthalein. This indicator, introduced by Sörensen [1908], changes into a deep blue with alkali, a change which appears to afford a sharper end point in the presence of the pigments of the serum than that of phenolphthalein. All the experiments were carried out in duplicate.

The presence of auxo-lipase in serum.

In order to convince myself of the existence of auxo-lipase in serum, I carried out a large number of preliminary experiments with sera and pancreatic extracts obtained at different times. The reaction was found to be remarkably constant, and the following experiment, chosen at random from many, illustrates the result obtained.

A pancreatic extract was prepared by mixing 2·5 kg. of finely minced pig's pancreas with 5 litres of glycerol. The mixture was strained through two layers of muslin after 24 hours. Before use one part of this extract was diluted with two parts of water. 1 cc. of this diluted extract, which will be designated as "lipase" in the following, was used in each experiment, the serum being added next and the fat emulsion last. The mixture was incubated for 17–24 hours, this period being chosen for convenience sake. In the following table the effect due to auxo-lipase is shown in the last column, expressed in cc. N/10 KOH[1].

[1] The amounts of N/10 KOH required by the pancreatic extract and by the emulsion alone have in all cases been deducted from the results given in this and the following tables.

Lipase cc.	Serum cc.	Emulsion cc.	Water cc.	N/10 KOH cc.	Effect of Auxo-lipase
1	–	3	3	13·3	—
1	1	3	2	27·1	13·8
1	2	3	1	36·7	23·4
1	3	3	–	37·3	24·0

When using the same amounts of serum and pancreatic extracts of different origin and incubating for the same time practically identical figures were obtained, the variations not exceeding ± 1 cc. N/10 KOH.

In the following series of experiments a smaller amount of pancreatic extract was taken and increasing amounts of serum from 0·2 cc. to 3·0 cc. were added. The results are shown in the following table.

Lipase cc.	Serum cc.	Emulsion cc.	Water cc.	N/10 KOH cc.	Effect of Auxo-lipase
0·5	—	3	3·5	5·9	—
0·5	0·2	3	3·3	17·7	11·8
0·5	0·4	3	3·1	20·8	14·9
0·5	0·6	3	2·9	22·4	16·5
0·5	0·8	3	2·7	24·9	19·0
0·5	1·0	3	2·5	28·2	22·3
0·5	1·5	3	2·0	31·9	26·0
0·5	2·0	3	1·5	33·2	27·3
0·5	3·0	3	0·5	35·8	29·9

Fig. 1 (p. 56) gives a graphic representation of the increase of the auxo-lipoclastic effect produced on the addition of increasing amounts of serum.

The auxo-lipase of serum is not only able to accelerate the action of active lipase but also to activate inactive lipase. The few observations which I have made in confirmation of Rosenheim's [1910] statements, were carried out with a sample of inactive lipase prepared by Dr Umeda in this laboratory. 10 mg. of inactive lipase suspended in 1 cc. of water, by itself practically without any action on olive oil, produced together with 1 cc. of co-enzyme an effect nearly equal to that of 1 cc. of my own diluted pancreatic extract. The following table shows the results produced on the addition of 2 cc. of serum to 10 mg. of inactive lipase.

Inactive Lipase 10 mg. in 1 cc.	Serum I	Serum II	Serum III	Co-enzyme	Lipoclastic effect cc. N/10 KOH
1	–	–	–	–	0·3
1	–	–	–	1	10·7
1	2	–	–	–	28·1
1	–	2	–	–	27·4
1	–	–	2	–	28·7

Not only has the addition of serum activated inactive lipase, but the effect produced is even greater than that of the co-enzyme.

These experiments leave no doubt about the existence of the auxo-lipoclastic action of serum. It may be mentioned, however, that in order to observe this reaction, it is necessary that enzyme, serum, and zymolyte shall be mixed in the order given. The following experiment illustrates the effect produced by an alteration in the order of mixing. In experiment (*a*) lipase and serum were mixed before the addition of the fat emulsion, whilst in

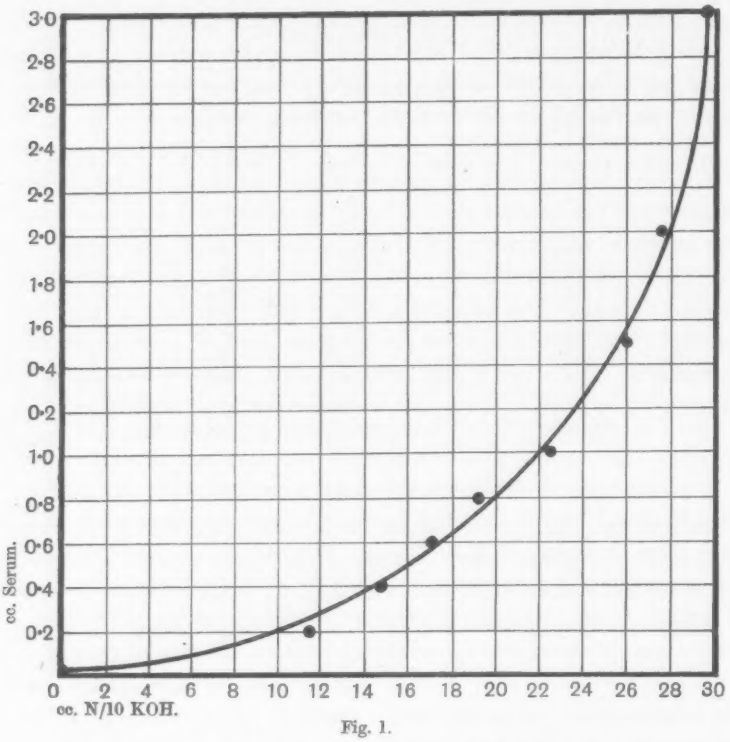

Fig. 1.

experiment (*b*) serum was added to the mixture of lipase and fat emulsion. The mixtures were brought to an equal volume by the addition of water and incubated for 24 hours.

(*a*)				(*b*)			
Lipase cc.	Serum cc.	Emulsion cc.	Effect of Auxo-lipase	Lipase cc.	Emulsion cc.	Serum cc.	Effect of Auxo-lipase
1	1	3	14·6	1	3	1	6·7
1	2	3	26·1	1	3	2	10·2
1	3	3	27·8	1	3	3	12·7

This experiment clearly demonstrates that by altering the order of mixing, the action of the auxo-lipase is diminished by over 50 %. The significance of this fact for the explanation of the reaction will be discussed later.

The stability of auxo-lipase.

In order to test the stability of the auxo-lipoclastic substance contained in serum, its effect was estimated in fresh serum and in portions which had been kept for over a month with and without toluene. The serum kept without a preservative had become putrid.

No difference was found between the action of fresh serum and that kept under toluene. To save repetition the figures are not given. The auxo-lipase in putrefied serum, on the other hand, had not only not been destroyed, but even slightly increased. In this and the following experiments 1 cc. of lipase and 3 cc. of emulsion were used and the mixtures brought up to equal volume by the addition of water. The necessary corrections were made as before. The figures were:

Putrefied serum cc.	Effect of Auxo-lipase
1	16·0
2	23·4
3	27·2

This increase may be due to concentration of the serum owing to evaporation, as it had been kept in an open flask in order to encourage putrefaction. These experiments confirm the results previously obtained in this laboratory that serum retains its auxo-lipoclastic activity even when kept for many months.

The heat-resistance of auxo-lipase.

This was tested by keeping undiluted serum for 0·5 hour in a water-bath at a temperature of 60°, and another portion, which had been diluted with an equal volume of water, for 10 minutes in a boiling water-bath. The various sera were tested in the usual way. The results are given in the following table, showing the effect of the auxo-lipase contained in 1, 2 and 3 cc. of serum, expressed in cc. N/10 KOH.

Fresh serum	Serum kept at 60° for 0·5 hour	Diluted serum	Diluted serum kept at 100° for 10 minutes
13·8	14·2	4·6	9·1
23·4	25·9	8·3	24·8
24·0	28·0	18·4	29·5

Auxo-lipase is therefore not destroyed by heating to 60° or 100°, but its action is even increased considerably. The significance of this fact and its bearing on the relationship of antitrypsin and auxo-lipase will be discussed later.

The effect of the removal of the proteins.

It was hoped to obtain more information about the nature of the auxo-substance by the removal of the proteins, which was effected (*a*) by heat coagulation in the presence of acetic acid, (*b*) by alcohol, and (*c*) by means of colloidal iron. In each case, however, the active substance was either carried down together with the protein or rendered inactive, and further the protein-free filtrates exerted even an inhibitory action on pancreatic lipase.

(*a*) *Heat coagulation in presence of acetic acid.* It was found that the presence of acetic acid did not interfere with the reaction at 37°, but that when the serum had been heated to 100° after the addition of acetic acid, its accelerating action was converted into an inhibition of the lipase.

A few drops of glacial acetic acid were added to 25 cc. serum, until an acid reaction to litmus was distinct.

Quantities of 2 cc. and 3 cc. were measured into a series of flasks. One series was kept for 10 minutes at 100° in a water-bath. The action of heated and unheated acidified serum was compared with that of normal serum in the usual way. The figures represent the increase or decrease (expressed in cc. N/10 KOH) of the lipoclastic action of 1 cc. of lipase after the addition of 2 and 3 cc. of the sera.

Normal serum	Serum + acetic acid (unheated)	Serum + acetic acid (heated for 10 minutes at 100°)
+16·4	+17·8	−5·5
+21·1	+21·2	−6·0

It seemed to be interesting to analyse the inhibitory action of the heated acidified serum further and to investigate whether the auxo-lipase was actually destroyed or if it was possible to restore its action by subsequent neutralisation.

25 cc. of serum were diluted with an equal volume of water, acidified with a few drops of acetic acid and kept at 100° for 10 minutes. The coagulated protein was filtered, finely ground in a mortar, boiled up with distilled water and again filtered and washed. It was then suspended in 50 cc. of water and one half of it neutralised with N/10 KOH.

The combined filtrates were concentrated, made up to 25 cc. with water and one half of the solution was neutralised with N/10 KOH.

The following results were obtained when testing the action of 2 cc. and 3 cc. of these fluids on lipase as before.

Protein suspension		Filtrate	
Washed	Neutralised	Acid	Neutralised
−6·4	+ 7·0	−6·0	−1·2
−5·7	+10·8	−5·8	−0·9

It will be seen that both the coagulated protein and the filtrate acquire an inhibitory action, when serum is heated in the presence of acetic acid. It seems further that the auxo-lipase is carried down with the coagulated protein, and is set free again by careful neutralisation, whilst the neutralised filtrate still remains inhibitory.

It may be mentioned in this connection that I have found in some preliminary experiments that milk also contains a substance which accelerates lipoclastic action. In this case also it is carried down by acetic acid with the caseinogen of milk, and set free again by the addition of a small amount of alkali.

(*b*) *Removal of proteins by alcohol.* 40 cc. of serum were poured into 400 cc. of boiling absolute alcohol, kept on a water-bath for half an hour and filtered. The coagulated protein was freed from alcohol by drying at 40°, finely powdered and divided into four parts. One part was suspended in 20 cc. of water. Another part was suspended and boiled with 20 cc. of water and finally to another part, suspended in 20 cc. of water, a few drops of N/10 KOH were added so as to make its reaction faintly alkaline to litmus.

The alcoholic filtrate was evaporated to dryness on the water-bath and the residue taken up with 200 cc. of absolute alcohol. After filtration the alcohol was evaporated and the residue taken up with 40 cc. of water.

The action of the various fractions on lipase was tested as before, 1 cc. and 2 cc. of each fraction being used. The results are given below.

Alcohol-Coagulum			
Suspended	Boiled	Neutralised	Alcohol Extract
+1·0	+3·4	+ 7·2	−0·1
+4·7	+7·6	+20·8	+0·7

Here again we see that the auxo-lipase is carried down with the coagulated protein, but in distinction from the acetic acid coagulum it retains its activity. Further, in analogy with its behaviour in the original serum, its activity is increased by boiling and still further by the addition of a small quantity of

alkali. The protein-free alcoholic extract is practically inactive. The insolubility of auxo-lipase in absolute alcohol differentiates this substance from the co-enzyme of lipase contained in watery pancreatic extracts.

(c) *Removal of proteins by colloidal ferric hydroxide.* By the use of this method [Michaelis and Rona, 1908] every trace of protein is removed from serum in the cold, without the introduction of any foreign substance into the filtrate. To 25 cc. of serum, diluted with 250 cc. of water, 20 cc. of colloidal ferric hydroxide solution were gradually added. The precipitated colloids were filtered off and washed until the wash-water was free from chlorides. The clear filtrate and wash-water were evaporated to 25 cc. The solution, containing the electrolytes and crystalloids of serum, was found to possess an inhibitory action on lipase.

When tested in the usual way the inhibitory action (expressed in cc. N/10 KOH) was: for 1 cc., — 0·5, and for 2 cc., — 1·1.

Evidently the auxo-lipase is removed together with the proteins by colloidal iron[1], a fact which speaks in favour of the colloidal nature of this substance. In order to test this still further, the behaviour of the auxo-lipase on dialysis was investigated.

The behaviour of auxo-lipase on dialysis.

As all the experiments which I carried out in this direction gave identical results, it will be sufficient to describe one of them in full. 30 cc. of serum were dialysed for seven days in a parchment diffusion shell (Schleicher and Schüll), toluene being used as a preservative. The distilled water was changed twice daily until it was free from chlorides. The dialysate was concentrated to 30 cc.

The dialysed fluid, which measured 48 cc., was divided into two parts. One half was used as such, whilst the other half was centrifugalised in order to test the action of the euglobulin fraction separately. Further, one part of the centrifugalised fluid, containing the serum albumin and pseudoglobulin, was heated on a water-bath for 10 minutes. The various fractions were tested as before, 1 cc., 2 cc. and 3 cc. being used of each and allowance being made for the dilution of the serum during dialysis. The results are given in the following table, the increase or decrease of the lipoclastic action being expressed in cc. N/10 KOH.

[1] The colloidal precipitate was not tested, as the large amount of iron in it was expected to render the result inconclusive.

Original serum	Dialysed serum	Dialysate	Albumin + Pseudoglobulin		Euglobulin
			Unheated	Heated	
+13·8	+16·4	−3·2	+18·4	+21·1	+2·0
+23·4	+21·3	−3·8	+21·4	+28·3	+4·2
+24·0	+24·1	−6·5	+24·4	+32·2	+4·3

The colloidal nature of the auxo-lipase is strikingly confirmed by this experiment, the action of the dialysed serum being little different from that of the original serum. Here again we meet with a characteristic difference between the behaviour of auxo-lipase and the co-enzyme of lipase, which diffuses easily [Umeda, 1915]. The experiments on fractionation of the serum proteins, as far as they go, point to an association of the auxo-lipase with the albumin and pseudoglobulin fraction. I was unable, owing to lack of time, to complete the fractionation.

The inhibitory action exerted by the dialysate suggests the question whether the inhibition is due to a substance of organic nature, or whether the electrolytes are responsible for it. As it has been shown by Umeda [1915] that the inorganic constituents of the co-enzyme of lipase are largely concerned in the activation of inactive lipase, an investigation of this question was likely to throw further light on the question of the identity of auxo-lipase and the co-enzyme.

The action of the mineral substances of serum.

The mineral substances remaining after the incineration of the whole serum as well as those of the dialysate were examined. 10 cc. of serum and 10 cc. of the dialysate (see above) were incinerated, the usual precautions being taken to avoid loss. The ash was in each case taken up with water and neutralised with N/10 HCl, using methyl-red as an indicator. The solution was evaporated to dryness, in order to drive off any excess of hydrochloric acid and the residue made up to 10 cc. with water. The following table shows the results, expressed in cc. N/10 KOH. For comparison the results obtained from the dialysate before incineration are added.

	1 cc.	2 cc.	3 cc.
Dialysate	−3·2	−3·8	−6·5
Ash of dialysate	−1·1	−1·7	−4·8
Ash of serum	−2·5	−2·9	−4·2

From these results it would seem that a large part of the inhibitory action of the dialysate is due to its mineral constituents, but that a certain amount of it is of an organic nature and lost by combustion. In sharp distinction

from the inorganic constituents of the co-enzyme of lipase, those of the serum not only fail to produce an accelerating effect but even inhibit lipoclastic activity, thus furnishing further evidence against the identity of auxo-lipase and co-enzyme.

The effect of serum lipoids.

In view of the fact that auxo-lipase is non-dialysable the possibility of its lipoid nature had to be considered. It has already been shown above, that an alcoholic extract of serum is practically inactive. I have further investigated the solubility of auxo-lipase in ether. For this purpose I made use of a serum powder obtained by drying serum in Petri dishes at 40° by means of an electric fan. I have convinced myself that a solution of this powder possesses the same effect as the original serum. 2 g. serum powder, corresponding to 20 cc. serum, were extracted in a Soxhlet apparatus with ether for four days. The residue of the ether extract was suspended in 20 cc. of water and tested in the usual way. Its effect on lipase (expressed in cc. N/10 KOH) was: 1 cc., 0; 2 cc., − 1·8; 3 cc. − 2·6.

This result together with the negative result produced by the alcohol extract shows that auxo-lipase is not of lipoid nature.

The relationship of auxo-lipase to antitrypsin.

It has already been mentioned above that the well-known presence of antitrypsin in serum has been suggested as affording an explanation of the auxo-lipoclastic action of serum. According to this view antitrypsin by inhibiting the action of trypsin prevents also the supposed destructive action of this enzyme on lipase.

The above described experiments on the heat resistance of auxo-lipase (p. 57) make it impossible to accept this view. According to Cathcart [1904] antitrypsin of serum—the albumin fraction—is destroyed on boiling and "is rapidly injured by heating. A temperature of 55° for half an hour suffices both in the presence and absence of alkali to destroy to a large extent the anti-action." Further, Hedin [1906] showed that it is readily destroyed at blood-heat by dilute acetic acid. In the above described experiments serum was kept at 60° for 0·5 hour and at 100° for 10 minutes, under which conditions antitrypsin is undoubtedly injured and destroyed. In spite of this the effect of the auxo-lipase was found in both cases considerably increased. Further, the action of auxo-lipase was not affected by dilute acetic acid at blood temperature.

Further experimental evidence against the assumed interaction of trypsin, antitrypsin and lipase is furnished by the following observation. Diluted pancreatic extract (1 : 3) was incubated for 24 hours, toluene having been added. The solution had acquired a powerful tryptic action which was found on comparison to be equal to that of a 1 % solution of Kahlbaum's trypsin. Under these conditions the lipase of pancreatic juice is entirely destroyed. It was therefore to be expected, if the lipase of pancreatic extract behaved like that of pancreatic juice, that the subsequent addition of serum to incubated pancreatic extract would fail to produce any effect, if its action were merely due to the antitrypsin contained in it. The following results were obtained on comparing the activity of freshly diluted and of incubated pancreatic extract. The effect due to auxo-lipase is expressed in cc. N/10 KOH.

Pancreatic extract				
Freshly diluted	Incubated for 24 h.	Serum	N/10 KOH cc.	Auxo-lipase
1	–	–	9·6	—
–	1	–	10·3	—
–	1	1	29·2	18·9
–	1	2	34·8	24·5

It will be seen that in distinction from the lipase of pancreatic juice, that of pancreatic extract is in no way affected by incubation for 24 hours, in spite of the development of trypsin. This protection may possibly be due to the presence of glycerol. Further, on addition of serum the action of auxo-lipase is even somewhat more strongly marked than in the case of freshly diluted extract (see p. 55).

Discussion.

The experimental material now available enables us to define the general properties of auxo-lipase somewhat more closely and to compare them with those of the co-enzyme of lipase.

Auxo-lipase of serum is thermostable, non-dialysable, insoluble in absolute alcohol and ether, and destroyed by incineration. It is associated with the serum-proteins and carried down with them by alcohol, by heat coagulation or by colloidal ferric hydroxide. Of the serum proteins the albumin and pseudoglobulin fraction appear to have a greater affinity to auxo-lipase than the euglobulin fraction. It is also noteworthy that serum contains an organic anti-lipoclastic substance the action of which is, however, masked by that of the auxo-lipase.

From its inability to pass through a dialysing membrane, one may conclude that auxo-lipase is of a colloidal nature, a conclusion which is strengthened by its behaviour towards colloidal ferric hydroxide.

This property sharply differentiates it from the co-enzyme of lipase, which dialyses easily and the activity of which, moreover, is not greatly diminished by incineration. The experimental evidence seems therefore to justify the conclusion that the auxo-lipase of serum and the co-enzyme of lipase are not identical.

With regard to the relationship of auxo-lipase and antitrypsin my experiments make it unfortunately impossible to accept the very plausible explanation advanced by Mellanby and Woolley. It may further be pointed out that from the experimental work of Shaw-Mackenzie [1912, 1913] it follows clearly that the antitryptic index of serum does not run parallel to its content in auxo-lipase and that, for instance, in the same individual a high antitryptic index may be associated with a low auxo-lipase content or a high auxo-lipase content of the serum associated with a low antitryptic index.

In dealing with this complex reaction the important factor of the physical condition of enzyme and zymolyte must not be lost sight of. As both these are insoluble, the presence of a colloidal solution like serum may influence the degree of emulsification of the zymolyte (fat) or the colloidal suspension of the enzyme (lipase).

From the experiment recorded above, however, in which the importance of the order of mixing was emphasised, we may conclude that this reaction is due to a direct action of the auxo-lipase of serum on the enzyme.

The expenses of this research have been defrayed out of a grant made to Dr Rosenheim from the Government Grant Committee of the Royal Society.

Conclusions.

1. The auxo-lipase of ox-serum is non-dialysable, thermostable and destroyed by incineration.
2. The auxo-lipase of serum and the co-enzyme of lipase are not identical.
3. No relationship could be demonstrated between the antitrypsin and the auxo-lipase of serum.

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VII. A SIMPLE HYDROGEN ELECTRODE.

By HENDRIK PIETER BARENDRECHT.

From the Laboratory of the Netherland Yeast and Spirit Manufactory, Delft, Holland.

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For methods to estimate the true reaction of a liquid biologists are indebted to a series of investigators, of whom only Sörensen [1909], Hasselbalch [1911, 1913], Michaelis [1909, 1912] and Walpole [1913] may be mentioned. The last of these has succeeded in constructing hydrogen electrodes, which allow very rapid and easy estimations.

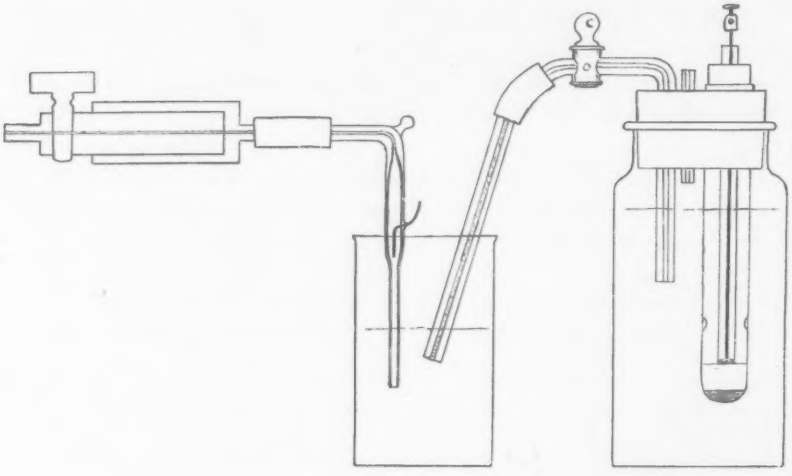

Fig. 1.

However, the apparatus, which Walpole used, is not simple and strong enough for continuous use and does not give all the security which is obtainable.

In the accompanying Figure 1 a modified arrangement is represented, which has proved still more serviceable for everyday work on the open bench without a constant temperature bath.

The hydrogen electrode is here simply a bent piece of glass tube (Jena glass), with a platinum wire sealed in its wall. The form of this glass tube, as will be shown further on, is carefully devised to meet all the requirements brought out by previous investigations. By means of a piece of rubber tubing it is connected to a perforated brass or copper syringe.

In carrying out an estimation, the hydrogen is first admitted through the tap, while the piston is still pushed home. After shutting the tap, the gas is nearly all contained in the electrode tube itself. By drawing the piston out and in several times, equilibrium between the enclosed hydrogen and the

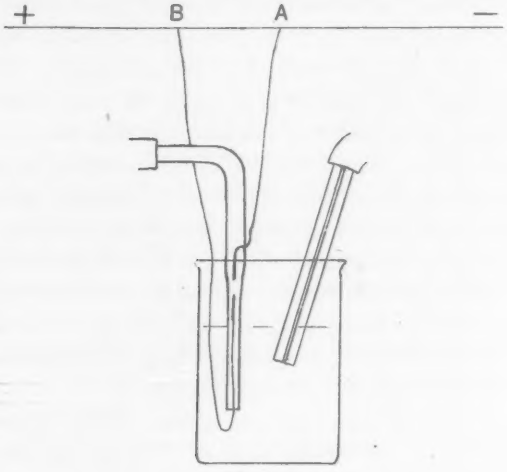

Fig. 2.

carbonic acid, which the liquid may contain, is better secured with this arrangement than with the apparatus of Walpole, where there is a considerable dead space behind the electrode tube. The sample, which is at last drawn up into the tube, when the gas-equilibrium is established, is evidently a fresh one.

Michaelis found, that a definite potential was much sooner attained, if the electrode only just dipped into the liquid. Konikoff [1913] drew attention to the fact, that dissolved oxygen might combine with the hydrogen of the electrode, which explained the fact observed by Michaelis. By submerging only a small point of the electrode a lasting saturation with hydrogen is sooner and better secured.

As the liquids to be examined in a biological laboratory often contain carbonic acid and oxygen, dissolved or loosely bound, it is important to take account of both of them.

As long as the liquid near the electrode contains some oxygen, this combines readily under the influence of the platinum black with the hydrogen of the electrode, thereby lowering its solution tension, i.e. its potential.

To secure a quick and automatic removal of the oxygen from the actual sample in the electrode tube I first tried to bring into this tube a second piece of blacked platinum wire, not connected with the electrode.

Figure 2 illustrates the experiments, made with this arrangement. Only the hydrogen electrode and the stretched wire AB, which served as potentiometer, are represented here. A is the point where the slide, connected with the gas cell, compensates the current from the accumulator. If now, before repeating the experiment with the same liquid, we bring inside the electrometer tube a platinum or gold wire with some platinum black on it, the other end of the wire coming out free into the air, the electrometer shows a large current, if the slide is pressed down in point A. Generally the slide has to be moved a considerable distance to the left (positive side), seldom to the right. If, however, we press the free end of the second wire, insulating it from the fingers with a piece of rubber tubing, on the potentiometer wire to the left of A, a point B can be found, at which the influence of the second wire is eliminated. This equilibrium does not last long. To maintain it we must gradually move the point B further to the left.

The interpretation of these facts is as follows. Ionised hydrogen can pass freely from the one blacked wire to the other. The hydrogen in the platinum black of the second wire will soon be oxidised by the oxygen, dissolved in the liquid, to which it is on all sides exposed; its solution tension will be lowered and it will therefore take more and more hydrogen out of the electrode. This transport of hydrogen is stopped, if we bring the second wire to a potential so much higher than that of the electrode, that the difference in solution tension of the hydrogen in the two wires is just compensated. The progressive oxidation of the hydrogen in the second wire will require a gradually increasing potential difference to do this.

If we pass hydrogen again through the electrode tube, while the second wire is inside it, both blacked wires will be saturated with hydrogen and therefore show no potential difference. This was observed repeatedly. Shortly after a renewed passage of hydrogen the point B came very near to A and then had gradually to be moved again to the left.

After saturating the liquid in the beaker (Sörensen's phosphate mixtures were mostly used in these experiments) with oxygen, *B* must be moved still more quickly away from *A*.

A blank platinum wire, introduced into the electrode tube, gives no effect at all, if its other end comes out free into the air. Neither does a gold or copper wire. If the other end is pressed on the potentiometer wire, very irregular effects are obtained by polarisation.

By these experiments it became clear, that the necessary removal of the oxygen from the liquid near the electrode wire had to be performed by the blacked platinum electrode itself.

I therefore placed the electrode as indicated in Fig. 1 with its point near the narrow part of the tube. If now, after securing the carbonic acid equilibrium as described in the beginning, the surface of the actual sample of liquid to be examined is several times drawn up and down the blacked wire (not higher and not lower), it is quickly reduced by the hydrogen in the relatively large surface of blacked platinum wire. The diameter of the platinum wire is about 0·6 mm., the inside diameter of the tube around it about 4 mm. By then pushing the reduced liquid down the long narrow end of the electrode tube until its surface is near the platinum point, we prevent original, not reduced, liquid from diffusing into the neighbourhood of the electrode.

With this arrangement it did not prove absolutely necessary to make the liquid just touch the electrode, though it is quite as simple and safer to observe this precaution.

These operations for securing the equilibrium of the carbonic acid and the removal of the oxygen take only a very short time, less than a minute.

Before this improvement was worked out, an increase of a few millivolts was generally noticed in the potential during the first minutes. The fact, that freshly boiled liquids did not show this phenomenon, drew attention to the marked influence, above described, of oxygen, often even of some dissolved air.

The glass bead on the electrode tube serves to fasten the thin copper wire, which is soldered to the outer end of the platinum wire.

The other half of the galvanic cell, represented in Fig. 1, has also proved to be very useful. A bottle of some 150 cc., filled with saturated KCl, is closed by a rubber stopper, through which three tubes pass. The wider one is the mercury-calomel-saturated KCl electrode proper, a tube, closed at the bottom, but with two openings in its side. The second is a capillary tube

with a tap and connected by a small piece of rubber tubing to a capillary, filled with cotton wool. The third tube serves simply to prevent air-pressure in the top of the bottle from causing inconvenience. This electrode bottle has been in use for months without needing any refilling or cleaning. The tap, which is not greased, remains always closed. There proved to be sufficient conductivity and even some capillary transport of KCl solution down the open cotton wool tube, purifying automatically the open end of it, which has dipped in the examined liquid; usually, in the morning, some KCl was found to have precipitated round the opening. Both glass tubes, dipping in the titrated liquid, may of course have smaller dimensions if the available volume is very small. The usual capacity of my electrode tube was about 0·3 cc.

SUMMARY.

A simple arrangement for the estimation of the true reaction of liquids, which remains always ready for use, is described and explained. It is in some points an improvement on Walpole's electrometric titrating apparatus for the open bench, special provision being made for allowing instantaneous and accurate estimations even in liquids, which contain carbonic acid and oxygen.

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VIII. ON THE CAUSE AND SIGNIFICANCE OF AN ABNORMAL REACTION OBTAINED IN TESTING URINE FOR SUGAR WITH FEHLING'S SOLUTION.

By W. CRAMER.

From the Physiology Department, University of Edinburgh.

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The investigations recorded here were carried out as the result of observations made on a urine obtained accidentally from the Infirmary. The urine was supposed to contain glucose and did, in fact, give the usual tests for glucose. Nylander's test was positive; with phenylhydrazine typical glucosazone crystals were obtained; it fermented and it showed a dextrorotation indicating a percentage of 1·5 % sugar calculated as glucose. But when Fehling's test was applied, a curious and striking abnormality was observed. On following the usual routine method of heating to boiling point, in two separate test tubes, equal quantities of urine and of half diluted Fehling's solution and pouring the two hot solutions together a sudden change took place at once: a deep scarlet-red transparent solution was obtained. If this solution was observed in transmitted light without further heating, it began to darken after about ten seconds, at first slightly, then more rapidly. At the same time the solution became less and less transparent and eventually after about a minute a very fine black precipitate appeared, which remained suspended in the fluid. The precipitate was so fine that on filtering it passed partly through the filter paper. If the mixture was allowed to stand for 24 hours, the precipitate became partly aggregated in soft slimy masses, which settled out at the bottom of the vessel. It could then be removed by filtration.

In studying the conditions under which the reaction took place it was noted that it was possible to prevent this abnormal reaction from taking place by simply diluting the urine with an equal volume of water. If Fehling's test was applied to such a diluted urine the typical result—a yellow or red precipitate—was obtained.

If Fehling's solution and the undiluted urine were mixed in the cold and then heated together to boiling point (instead of heating them separately and mixing them when hot) a red precipitate was formed at first, which on further heating gradually darkened. This darkening became less and less marked when the relative amount of Fehling's solution with which a given volume of urine was mixed, was increased. The opposite condition—using an excess of urine and relatively little Fehling's solution—produced again a darkening of the cuprous oxide which was formed at first. If the red cuprous oxide obtained by heating together an excess of Fehling's solution and relatively little urine was added to a further quantity of the urine, and the mixture heated, the red precipitate again showed a darkening. It is perhaps necessary to state that the darkening of the fluid is quite different from that due to the action of alkali on sugar and that it is not due to the caramelisation of the sugar.

On investigating the nature of the black precipitate, it was thought at first from its physical appearance to be of an organic nature, perhaps a combination of copper with an abnormal organic constituent of urine. It was found, however, that the precipitate was not of an organic nature, for on heating weighed quantities of the precipitate in a crucible, it showed an increase in weight and formed a black mass, different in appearance from the original black precipitate and identical in appearance with cupric oxide, which, in fact, it was, as it yielded copper by being heated in a current of hydrogen. On treating the original black precipitate with acids, it was found that it dissolved readily in nitric acid with the evolution of brown fumes and formed a clear green solution: it was not readily affected by hydrochloric acid or sulphuric acid. It behaves therefore in the same way as metallic copper and is, in fact, copper in an extremely fine state of division, finer even than the copper obtained by reducing a cupric salt by means of a watery solution of glucose as described below. If the filter paper which has been used for the filtration of the precipitate is allowed to dry in the air, it acquires the characteristic green colour of basic copper carbonate (verdigris).

The fluid from which the precipitate had separated out had in transmitted light sometimes a brown, sometimes a greenish-black appearance, while in reflected light it was black, thus suggesting that it represented a suspension of very fine particles of the material which furnished the black precipitate, in other words that it was a colloidal solution of copper. This was confirmed by the fact that hydrogen peroxide was decomposed when added to the warm colloidal solution.

The conclusion arrived at, that the black precipitate is finely divided metallic copper, is less surprising since I have found that Fehling's solution is readily reduced by glucose to metallic copper. It was thought, at first, that reduction to copper would take place only if cuprous oxide were present in colloidal solution, and that therefore the presence of such substances as creatinine, which are capable of holding cuprous oxide in solution, was necessary. But this is not the case.

If a hot concentrated solution of glucose, or solid glucose, is added in excess to boiling Fehling's solution, one notices as the first change the well-known formation of red or yellow cuprous oxide. If heating is now continued for one or two minutes, this red or yellow precipitate darkens and the whole mixture assumes a dark brown colour. If at that stage the flame is removed, so that boiling is interrupted, an evolution of gas will be seen to be proceeding. If boiling is continued for three minutes a reddish brown heavy precipitate is obtained which rapidly settles at the bottom of the vessel and which, when washed and dried in a steam bath, is finally obtained as a fine powder having the characteristic colour of copper. It dissolves in nitric acid with the evolution of brown fumes. This and the increase in weight obtained on heating show that the precipitate consists almost entirely of metallic copper admixed probably with a small amount of cuprous oxide, which has not been further reduced.

The increase of weight obtained on heating should be 25·1 per cent. if the precipitate were metallic copper and 11·2 per cent. if the precipitate were cuprous oxide. The following figures were obtained with three different preparations:

1. 0·4274 g. precipitate gave 0·5202 g. CuO. Increase in weight, 21·71 %.
2. 0·2479 g. „ „ 0·3056 g. CuO. „ „ 23·3 %.
3. 0·2385 g. „ „ 0·2954 g. CuO. „ „ 23·85 %.

The last preparation had been dried in a current of nitrogen, and heated in a current of oxygen. As a control the CuO obtained was reduced again by heating in a current of hydrogen.

0·2954 g. CuO gave 0·2362 g. Cu. Decrease in weight, 25·06 %.

The only difference which exists between the reduction produced by pure glucose solution and that brought about by this urine consists in the state of division. Since in the latter case one obtains at first a colloidal solution of cuprous oxide, which is then further reduced to a colloidal solution of copper, the black precipitate which one eventually obtains as the result of the "coagulation" of the colloidal copper solution represents a

much finer state of division of the metal than the typically copper-coloured precipitate obtained with glucose in watery solution.

The black precipitate obtained from the urine was, of course, not chemically pure metallic copper, as it was mixed with the phosphates which had separated out from the urine as the result of boiling it with the alkaline Fehling's solution. It was probably also contaminated with some organic material adherent to it. Owing to this fact the increase in weight obtained on heating weighed quantities of the dried precipitate varied considerably (from 10 % to 14 %) and was below the value calculated for pure copper (25·1 %), but in some cases above that calculated for cuprous oxide (11·2 %).

It is now easy to understand the abnormal reaction described at the outset of this paper. When the heated urine and Fehling's solution were mixed, the glucose present in the urine at once reduced the cupric oxide to cuprous oxide. This, however, remained in solution for reasons which will be discussed presently, and then produced the transparent scarlet-red solution that has been described. This colloidal solution was then further reduced by the glucose to colloidal copper, which at first formed a colloidal solution from which it was gradually precipitated to form the black slimy masses of finely divided copper.

The question now arises: why is this reduction to metallic copper by urine containing glucose so rare, as it evidently is, since it has never been described? The answer is to be found in the following facts. The urine had the colour of normal urine. On diluting it with an equal volume of water, it gave, as has already been stated, the typical reduction with the appearance of cuprous oxide. On inquiry at the ward it was found that the total volume of urine excreted by the patient was rather below than above the normal. Dr R. A. Fleming, under whose care the patient was, and to whom I am greatly indebted for his readiness to place the material at my disposal, has very kindly given me all the details concerning the clinical aspects of the case, from which the following statement has been abstracted.

The case was demonstrated by Dr Fleming at a meeting of the Edinburgh Medico-Chirurgical Society [1914].

"The patient was a young woman, age 22 years, weight 8 st. $3\frac{3}{4}$ lbs. She complained of 'weakness and tiredness' from which she had suffered for three years. Six months before admission to the hospital her doctor discovered sugar in the urine, for which she was treated by being placed on a strict diabetic diet.

The patient was very neurotic and frequently complained of 'Globus Hystericus' with palpitations on any excitement. She also had a degree of pharyngeal anaesthesia common in hysterical subjects. Apart from the excretion of sugar in the urine no other symptoms of disease were found.

The urine varied in amount from 24 oz. up to 52 oz., with an average of about 30 oz. in the 24 hours. The specific gravity of the urine varied from 1·030 to 1·035. There was no albumin in the urine. The amount of sugar varied greatly, from 0·4 grains per oz. and about 15 grains per day, to 5 grains per oz. and about 150 grains per day. Dietetic measures did not markedly affect the amount of sugar excreted. Apart from these measures she was treated also at first with codeine, later with a tonic containing strychnine. It was noted that whenever the sugar diminished the feeling of 'tiredness' was less marked.

After three months in hospital the patient gained 8 lbs. in weight and went out, feeling much better, although the excretion of sugar persisted."

Briefly summarised, the case is not a typical case of diabetes mellitus, but one of a marked glycosuria, unaccompanied by polyuria. At the time when these investigations were made, the urine contained about 1·5 % of glucose (estimated by the polarimeter). We have therefore to deal with a urine of normal concentration, in so far as the normal urinary constituents are concerned, which contains glucose in such amounts that more sugar is present than necessary to reduce all the cupric oxide, provided that the usual method of carrying out the test is applied (equal amounts of urine and half diluted Fehling's solution). Now if glucose is added to normal urine in such amounts that the urine contains about 1·5 % of glucose, and Fehling's test is applied to this urine, a deep red, transparent solution is obtained, which represents a colloidal solution of cuprous oxide. The appearance of this red solution instead of a precipitate of cuprous oxide is due partly at any rate to the presence of creatinine, which, as MacLean has shown [1907], is capable of holding cuprous oxide in solution. Other substances, perhaps the colloid material of urine acting as a protective colloid, also contribute to this effect. If this red solution is further heated it darkens to a deep brown solution and deposits a reddish brown precipitate, so that it is possible to imitate to a certain extent the abnormal reduction described at the outset of this paper simply by using a normal urine containing glucose in a certain concentration.

The change of colour from red to black is, however, not so striking in the case of normal urine to which glucose has been added. A much better imitation of the reaction can be obtained with normal urine in the following way.

Add to half a test tube full of normal urine two drops of chloroform, shake vigorously and apply Fehling's test at once. One obtains then the red, transparent, solution, which darkens without further heating and eventually deposits a black precipitate. If to urine which has been agitated with chloroform, and then allowed to stand so that the chloroform has time to settle, Fehling's test is applied, only the red solution is obtained which does not blacken on further heating. If now to this red solution sugar is added, blackening occurs.

It is therefore clear that this "abnormal" reaction is due to the presence in sufficient quantity of a reducing substance in urine of a normal concentration. The amount of the reducing substance present must be in excess of the amount necessary to reduce all the cupric salt used in the test.

The clinical interest which attaches to this reaction appears to be that it indicates the presence of relatively large amounts of sugar in a urine of normal concentration; it indicates, in other words, a marked glycosuria unaccompanied by polyuria.

Some years ago I obtained from the Infirmary a urine giving exactly the same reaction. Unfortunately, the patient left the hospital before I had time to investigate the condition. But as both this former and the present case were obtained accidentally, when material was being collected for teaching purposes, it seems likely that these "abnormal" reactions may occur much more frequently than would appear from the complete absence in the literature of any similar observation.

Since watery solutions of glucose are capable of reducing cupric salts to metallic copper, it is to be expected that urine from a typical case of diabetes mellitus, where we have a glycosuria accompanied by a polyuria, will also give the reduction to metallic copper. This is, in fact, the case, especially if excessive dilution be avoided by using Fehling's solution undiluted, or if Fehling's solution is diluted with an equal volume of normal urine. If after the appearance of the yellow or red precipitate of cuprous oxide boiling is continued, a marked darkening of the precipitate and of the solution occurs, varying from a light brown to the dark reddish brown copper colour, characteristic of the precipitate obtained by boiling Fehling's solution with a watery solution of glucose. It must be noted, however, that this further reduction occurs only when boiling is continued, and is even then not nearly so striking as the reaction described at the outset of this paper. The variations in colour depend on the amount of glucose in the urine, on the proportion of urine and Fehling's solution, and on the concentration of the mixture both as regards

the ordinary urinary constituents and the glucose. The darkening of the brick-red cuprous oxide precipitate must have been frequently observed, but it appears to have been ascribed generally to the caramelisation of the sugar by the alkali of Fehling's solution. That this is not the case is evident from the fact that exactly the same reaction is obtained, as stated above, if chloroform instead of glucose is added to the urine.

The reaction obtained with urine containing chloroform is also of interest, because it demonstrates that this "abnormal" Fehling's reaction is due mainly to the cuprous oxide dissolving power of normal urine. For chloroform shaken up with water gives with Fehling's solution only the red precipitate of cuprous oxide, which does not become further reduced to copper even after prolonged boiling.

SUMMARY.

1. A reduction of cupric salts to metallic copper can be brought about by concentrated watery solutions of the reducing sugars. It takes place even more readily when the reducing sugar is present in a urine of normal concentration with regard to the normal urinary constituents, being facilitated by the fact that some of the constituents of normal urine are capable of holding cuprous oxide in solution.

2. Clinically this abnormal reaction with Fehling's solution is of interest, as such a reaction may be taken to indicate a marked glycosuria unaccompanied by polyuria, and not a typical diabetes mellitus.

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IX. ON THE CHOLESTEROL CONTENT OF THE TISSUES OF GROWING RATS WHEN UNDER VARIOUS DIETS.

By PERCY EDWARD LANDER

Lindley Student of the University.

From the Physiological Laboratory of the University of London, South Kensington.

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It has for some time been known that growing animals are unable to thrive for any length of time on a diet consisting of a mixture of pure protein, fat, and carbohydrate. Stepp [1909, 1911] in a series of experiments on mice attempted to estimate the importance of lipoids in nutrition, and found that food mixtures after extraction with lipoid solvents could not maintain life, but that the extracted matter when added to the food made it once more efficient to do so; he could not obtain this result, however, by adding any known lipoid. A similar series of experiments has been carried out by Osborne and Mendel [1911] on rats. Their earlier work was directed to the question as to whether life could be maintained on a dietary containing a single individual protein, instead of mixtures of proteins such as ordinary normal dietaries comprise, and they were led to answer this question in the affirmative. Maintenance is possible so long as the protein stored is one not deficient in certain amino-acid groupings, but their earlier experiments led these authors to realise the importance of factors other than protein and energy supply and they found that synthetic dietaries which were able to maintain the life of fully grown rats were unable to do so with growing animals, but that a small addition of tissue extracts added to those synthetic diets induced normal growth.

In their later experiments these authors showed that the addition of milk products to the food promoted rapid growth in rats which had remained stationary in weight when on the original artificial diet. The material which was added they called "protein free milk"; it was prepared by removing

as far as possible by precipitation and coagulation, the proteins from fat free milk, the fluid being then evaporated at low temperatures and the residue ground to powder.

Hopkins [1912] in an extended series of experiments in which groups of young rats were fed upon artificial mixtures of isolated caseinogen, fat, carbohydrates and salts side by side with similar groups which were fed on the same basal dietary with the addition of a minute quantity of fresh milk, has found that in those experiments in which the basal diet contained fully purified material the rats without milk soon failed to grow. When the constituents were less completely purified then slow growth occurred. In all cases the milk addendum, although its total solids only amounted to 4 % or less of the whole food eaten, induced normal and continued growth.

The total energy consumption of these animals was determined and it was shown that the rats upon the purer dietary ceased to grow at a time when their intake was more than sufficient quantitatively to maintain normal growth. The absolute consumption of the animals growing rapidly with the milk addendum was greater than that of those growing slowly upon the basal dietary alone; but the consumption per unit of live weight was in comparable groups nearly the same. It was further shown in these experiments that if growth continued, as upon the less pure basal dietary, the small milk addendum reduced the food consumption necessary for a given weight increment to one half or less.

Cessation in increase of weight upon the pure dietary took place before any failure in appetite, although the consumption might, later, fall to a lower level. It was therefore suggested that any effect of the addendum upon appetite must have been secondary to a more direct effect upon growth processes.

Much discussion has taken place over the question as to what is meant by "growth." A living organism may increase its exterior dimensions without increasing its mass, and, on the other hand, the mass may be increased without any "growth processes" taking place.

When young rats are being fed on a normal diet they certainly grow, by which we mean that the amount of living material in their bodies increases and they increase in size permanently, but still we have to define our terms "grow" and "living material," and we find ourselves arguing in a circle.

The evidence now accumulated, however, points fairly to the conclusion that when "growing rats" are put on to a pure synthetic dietary the phenomena observed during their former growing period cease.

We may state what has happened either by saying that their "growth processes" have ceased, or by saying that they are being starved and hence cease to grow. It would seem that the latter more accurately states the case.

The synthetic diet seems to lack something which is necessary for the proper utilisation of the other food stuffs by the tissues of the animal.

We may compare the tissues of the rat and the food material to a heap of powder ready to unite on the application of a flame—the hormone present in a normal diet, but absent from a synthetic diet.

The fact then being fairly well established that "growth processes" cease when animals are fed on pure synthetic diets, or in other words, starved, it seemed to be of interest to carry out experiments to endeavour to determine how far variations of the cholesterol content of the bodies of growing rats occur, when fed on various dietaries, especially with regard to the question as to whether the growing organism can manufacture cholesterol when deprived of that substance in the food. It seemed that an answer might be obtained to this question, and to others bearing on the origin, fate and functions of cholesterol in the animal organism, by taking a number of sets of rats, from a common starting point—which was chosen as the period after birth at which the animals commenced to feed themselves—and estimating the total cholesterol content of the bodies of these rats at once, and then comparing the figures obtained with those from other sets of rats, which had been fed for definite periods on various dietaries of known composition. The original set of controls then gives us the normal quantity of cholesterol in the bodies of the rats when they start to feed themselves, whilst the numbers from the other sets give us an indication as to any increase or decrease in the quantity of cholesterol present.

It was determined at the outset to keep all the sets of rats on their particular dietaries until they had either doubled their weight or had been kept for a time equal to that in which a set of rats fed on ordinary bread and oatmeal had doubled their weight, which was found to be just over three weeks.

Owing, however, to the unpalatability of certain of the synthetic and alcohol-and-ether extracted diets, this was found to be difficult as the rats commenced to die after a certain time, so that in some cases I was unable to extend the observations beyond a period of about 14 days.

An endeavour was also made to find a synthetic diet which, by the addition of cholesterol and other substances such as cholesterol esters, lecithin, lemco, etc., would keep the rats in normal growth.

No synthetic diet was found satisfactory for this purpose and hence no reliable evidence is to hand as to how the cholesterol content of the rats would vary under such conditions.

Method. The animals employed in these experiments were ordinary tame white rats and were mostly bred in the laboratory. They were kept with the mothers until they were able to feed themselves and were then taken in sets of varying number and put into square wire cages of about eight cubic feet capacity. The bottoms of these cages were also of wire, the mesh being of sufficient size to allow the faeces to drop through into a trough placed beneath. Each cage had in it a large pad of cotton wool. It was not thought necessary for the purpose of the experiments to feed the animals separately; they flourish much better when not alone, and furthermore individual variations are eliminated. On the other hand, this plan is open to the disadvantage that if an animal dies the others commence to nibble it.

Diets employed. Altogether 13 sets of rats were analysed; the method of analysis being a modification of Windaus' digitonin process fully described by Fraser and Gardner [1910]. The details of the results are given in Tables I and II.

TABLE I.

Experimental set	Diet	No. of rats	Total weight in grams	Percentage of cholesterol			Weight of cholesterol per rat in grams			Average weight of rats
				Total	Free	Ester	Total	Free	Ester	
1	Starting feeding three weeks old	5	122·45	0·1467	0·0944	0·0523	0·0359	0·0229	0·0130	24·49
2	do. do.	6	133	0·1721	0·1373	0·0348	0·0380	0·0304	0·0076	22·20
3	Bread and oatmeal	4	142·4	0·200	0·144	0·056	0·071	0·051	0·020	35·60
4	Synthetic and milk salts	3	61·0	0·238	0·2122	0·0258	0·048	0·042	0·006	20·30
5	Synthetic and incinerated salts	1	26·8	0·136	0·136	nil	0·0366	0·0366	nil	26·8
6	Synthetic and mixed proteins	3	67·0	0·174	0·174	nil	0·0388	0·0388	nil	22·3
7	Synthetic and milk only	4	117·3	0·2162	0·1198	0·0964	0·0634	0·0351	0·0283	29·3
8	Synthetic and milk and cholesterol	3	76·6	0·5236	0·1608	0·3628	0·1340	0·0429	0·0911	25·50
9	Synthetic and cholesterol	2	34·6	0·428	0·4079	0·0201	0·074	0·0705	0·0045	17·3
10	do. do.	3	52·6	0·5046	0·4821	0·0225	0·0883	0·0812	0·0071	17·5
11	Synthetic and cholesterol esters	2	41·5	0·3606	0·3183	0·0423	0·075	0·066	0·009	20·75
12	do. do.	6	100·0	0·415	0·272	0·143	0·069	0·045	0·024	16·7
13	Ether extracted bread and oatmeal, and cholesterol	4	134·5	0·328	0·3178	0·0102	0·1104	0·1070	0·0034	33·60

TABLE II.

Expt set	Diet	No. of rats analysed	Average wt. of rat at beginning of experiment	Average weight of rat at end of experiment	Average increase in weight	Length of expts in days
3	Bread and oatmeal	4	18	36·7	18·7	25
4	Synthetic diet and milk salts	3	22·2	20·3	−1·9	16
5	„ „ „ incinerated salts	1	25·7	27	1·3	16
6	„ „ „ mixed proteins	3	20·7	22·8	2·1	15
7	„ „ „ milk only	4	18·2	30·2	12	24
8	„ „ „ milk and cholesterol	3	19·5	26·7	7·2	24
9	„ „ „ cholesterol	2	19·3	18·25	−1·05	13
10	„ „ „ „	3	20·0	18·3	−1·7	15
11	„ „ „ cholesterol esters	2	19·3	20·7	1·4	16
12	„ „ „ „	6	16·6	17·0	0·4	15
13	Ether extracted bread and oatmeal, and cholesterol	4	18·8	34·7	16	31

Set (1). This set consisted of 5 rats which were killed and analysed at the period when they were just commencing to feed themselves. They were then 22 days old, and weighed altogether 126·45 g., the average weight per rat being 25·29 g. The actual minced up material used was 122·45 g. This mass was pounded up with sand and plaster of Paris and allowed to dry. It was then extracted with ether in a Soxhlet apparatus for about 14 days. The ethereal extract was made up to one litre, one half of which was analysed at once, and gave by direct precipitation with digitonin in hot absolute alcohol 0·236 g. of the digitonin compound corresponding to 0·0573 g. of cholesterol. The other half was saponified and gave 0·3698 g. of the compound corresponding to 0·0898 g. of cholesterol. This represents a total cholesterol content of 0·1798 g. corresponding to 0·0359 g. of cholesterol per rat which may be taken as our control standard.

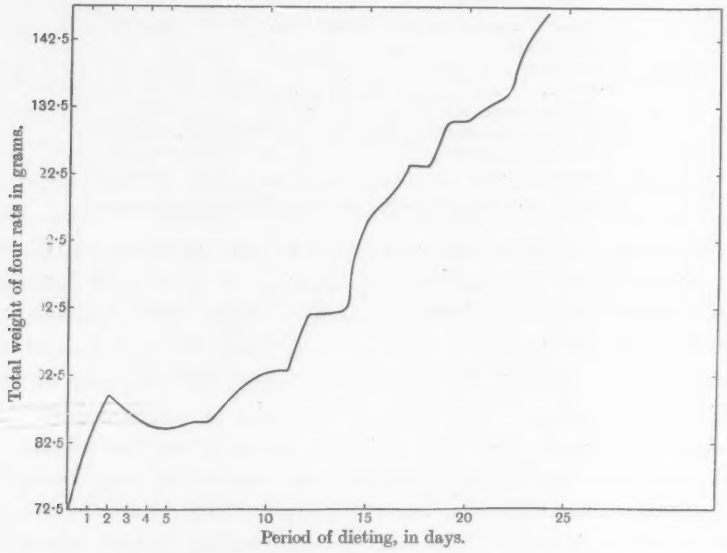
Set (2). This set, consisting of six rats, was treated in a similar way to the last. They were 24 days old and weighed 134·5 g., the average weight per rat being 22·4 g. The actual minced up material used was 133 g.

Total cholesterol content 0·2289 g., or an average of 0·038 g. of cholesterol per rat.

Set (3). *Ordinary diet.* This set consisted of four rats which commenced to feed themselves about 28 days after birth, when their total weight was 72 g. They were then put on a diet of bread and oatmeal and doubled their weight in 25 days. During this period they were given 377 g. of dry bread and 155 g. of oatmeal, the total being 532 g., 447 of which were eaten. The dry bread was soaked in water after being weighed and mixed with the oatmeal.

The total weight of the four rats when killed was 146·8 g., their average weight being 36·7 g., whilst the actual weight of material minced up was 142·4 g. Total cholesterol content 0·2846 g.; corresponding to 0·071 g. cholesterol per rat.

This diet of bread and oatmeal was analysed for phytosterol, 0·0235 % of total phytosterol being found. The method employed was to heat the food up with a 10 % solution of alkali on a water bath for some hours, and then after making acid with strong hydrochloric acid, to continue the heating for a similar period. The mass was then treated with ether and estimated



Curve 1. Four rats, ordinary diet (Set 3). Total weight curve.

in the ordinary way. We see now that the increase in the cholesterol per rat over set (1) is 0·071 — 0·0359 = 0·035. Therefore the total increase in cholesterol in the bodies of the four rats is 0·14 g.

Altogether 447 g. of food, containing 0·0233 % of phytosterol were eaten, which means that 0·105 g. phytosterol were ingested. Hence taking into consideration individual variations in the rats and possible errors of experiment there is here no evidence of any synthesis of cholesterol; all the phytosterol contained in the food seems to have been laid hold upon and stored. Curve 1 represents the growth of these rats.

Set (4). *Basal synthetic diet + milk salts.* This set consisted of six rats which were not started on the dieting until they were 28 days old, when they weighed 133·5 g., their average weight being 22·2 g. These animals were fed on the synthetic diet used by Hopkins [1912] in his extended series of observationson the growth of rats when fed on various diets, which consisted of pure protein, fat, and carbohydrate, with salts added of the same nature and percentage as those found in milk.

Composition of food.

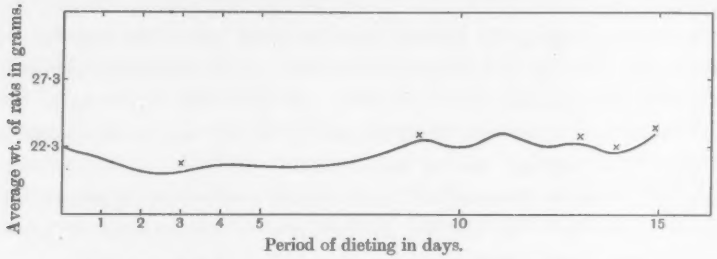
Protein (Hammarsten's pure casein)	22 %
Potato starch	42 %
Cane sugar	21 %
*Lard	12·4 %
Calcium phosphate	1·95 %
Magnesium phosphate	0·35 %
Potassium chloride	0·15 %
Sodium chloride	0·15 %

* The lard was carefully purified and was quite free from cholesterol.

By feeding the rats on this diet I wished to determine firstly whether any growth occurred or not, and secondly whether any increase of cholesterol occurred in the body, none being present in the food. Before administering the food, the protein, starch and sugar were mixed dry, and the lard was then carefully rubbed in by hand and the whole mixed together till of a uniform composition. Thus prepared the mixture represented the dry weight of the food. Each day's ration for the set of rats was weighed out dry, then water was added, a little at a time, the whole being thoroughly mixed so as to make the mass into a light paste, of such consistency that the rats would eat it freely. More food was always given than the rats would eat, and was placed in a shallow vessel so as to be easily accessible; water was placed in a separate vessel. It was found possible to determine the food eaten with great accuracy; the rats spilled out very little, and practically none of the faeces became mixed up with it. The food left over from the previous day was taken out each morning, before feeding, carefully dried and weighed.

During the period of dietary the rats were given altogether 211 g. of food, 175 g. of which were eaten, but in considering these figures one must remember that the rats died progressively, during the experiment, apparently owing to the unpalatability of the food, and by the end of the 16th day only one remained, two having died earlier in the day.

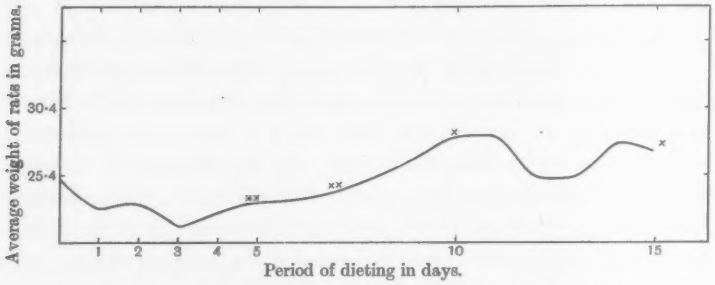
The average weight of these three rats was 20·3 g., and as the average weight of the rats at the commencement of the period of dieting was 22·2 g., we see that no growth has taken place. Curve 2 represents the growth curve of these rats, their average weight being plotted against days of dieting, and the small crosses represent the periods at which successive rats died.



Curve 2. Synthetic diet + milk salts (Set 4). Average weight curve.

Some difficulty was experienced in keeping the rats from nibbling those that died. No nibbling of any importance occurred in this and the next sets, but some did occur in those sets which were fed on an ether and alcohol extracted diet.

Total cholesterol content 0·1454 g., corresponding to 0·048 g. cholesterol per rat. We thus see that this figure is very near that found for set (1) and that no apparent increase of cholesterol has taken place during the period of growth.



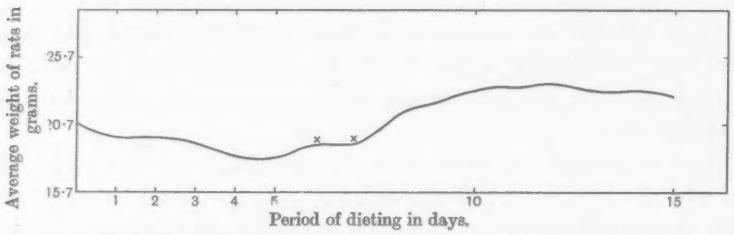
Curve 3. Synthetic diet + incinerated salts (Set 5). Average weight curve.

Set (5). This set also consisted of six rats taken from the same litter as the above set, and the rats were put on the special diet 28 days after birth. Their total weight at this time was 154 g., or an average weight of 25·7 g. They were fed on exactly the same diet as set (4) except that the salts used

were obtained from incinerating bread and oatmeal. During the period of dieting the rats were given 165·5 g. of food, 125 g. of which were eaten. As in the case of set (4) the rats did not thrive well on this diet, dying at intervals during the course of the experiment, until at the end only one rat was left weighing 27 g., so again we see that practically no increase in weight has taken place.

Curve 3 represents the growth curve of these rats. The last rat was killed on the 16th day and analysed, the actual weight of material used being 26·8 g. Total cholesterol found 0·0366 g. per rat. This figure again very closely approximates to that found for set (1), no increase in the cholesterol content of the organism having taken place.

Set (6). This set consisted of five rats which were fed on a pure synthetic diet as in the case of set (4), but half the protein was replaced by protein obtained from bulls' testicles.



Curve 4. Synthetic (mixed proteins) + lecithin (Set 6). Average weight curve.

This was obtained as follows:

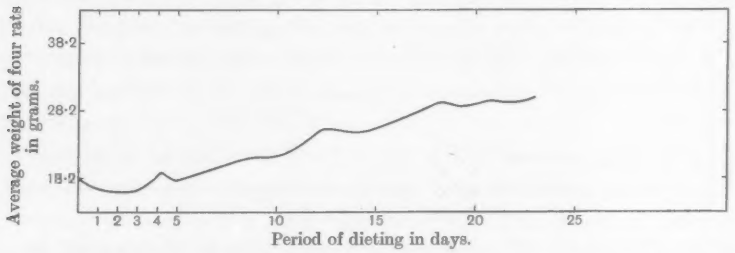
The tissue was carefully ground up and boiled with alcohol, added in the ratio of one litre of alcohol to 300 g. of tissue. The alcohol was filtered off and the boiling repeated. The tissue was then extracted with ether in a Soxhlet apparatus for a fortnight, after which it was again extracted by petroleum ether for a considerable time. In addition 0·5 % of lecithin prepared from bulls' testicles was added. The diet was entirely cholesterol free.

At the beginning of the experiment the five rats weighed 103·5 g., being an average of 20·7 g. One rat died on the 7th day and one on the 8th. On the 16th day, the remaining three rats weighed 68·5 g., being an average of 22·8 g. During this period they were given 150 g. of food, 130 of which were eaten. The actual amount of material analysed was 67 g.

Total cholesterol content per rat 0·0388 g.

Curve 4 shows average growth during the course of the experiment.

Set (7). This set consisted of four rats born on May 30, 1913, and the animals were put on their special diet on June 30, 1913. This diet was the same as the synthetic diet employed in sets (4) and (5); in addition to which 20 cc. of milk were given each day. We wished to see how the addition of milk alone to the synthetic diet would influence growth and whether the small quantity of cholesterol in the milk would be picked out by the organism and utilised for growth. The weight of the four rats at the commencement of the experiment was 75 g., corresponding to an average of 18·2 g. per rat. At the end of the experiment their total weight was 120·9 g., representing an average of 30·2 g. Each rat had therefore put on during the course of the experiment 12 g., which is a greater increase than in the following case in which cholesterol was also added to the diet, but not so great as in set (13), which had ether extracted bread and oatmeal and cholesterol. During the



Curve 5. Synthetic diet + milk only. No cholesterol (Set 7). Average weight curve.

period of dieting of 24 days, the rats were given 366 g. of food, and 480 cc. of milk, 265 g. of the food being eaten.

Milk contains about 4 % of fat, and milk fat about 0·5 % of cholesterol. The milk given to the rats would thus contain altogether about 0·096 g. of cholesterol.

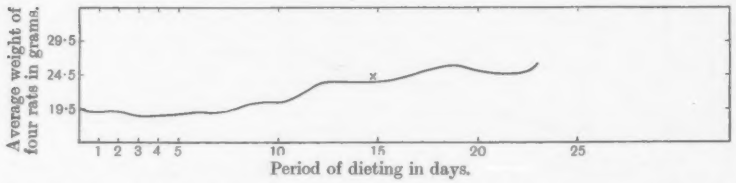
All the rats remained perfectly healthy during the course of the experiment, none having died. The rats were killed on July 22 and analysed, the actual weight of material used being 117·3 g. Total cholesterol found 0·252 g.; corresponding to 0·063 g. of cholesterol per rat. Now assuming that each rat had in its body the normal quantity (set 1) of cholesterol, viz. 0·0359 g., the total quantity would be 0·144 g. for the four rats. Therefore the total increase for the four rats is $0{\cdot}252 - 0{\cdot}144 = 0{\cdot}108$.

The milk given contained as we have seen about 0·096 g. of cholesterol altogether. These figures would therefore indicate that the cholesterol in the milk was greedily picked out and stored in the organism. All the cholesterol

found can thus be accounted for by that contained in the milk, and, taking into account the errors of experiment, there is no evidence of any synthesis having taken place. Curve 5 represents the average growth curve for this set.

Set (8). This set, consisting of four rats from the litter born on May 30, 1913, were put on their special diet on June 30. The diet was exactly the same as the last except that 2 % of cholesterol was added to the food and only 10 cc. milk given each day instead of 20. The dry food after being weighed was carefully mixed with water as in the case of set (4). The object of feeding the rats on this diet was to see how the addition of the milk affected growth, and whether, should normal growth occur, the cholesterol content of the tissues would approach or exceed the normal figure.

The weight of the four rats at the commencement of the period of feeding was 78 g., corresponding to an average of 19·5 g. During the period of dieting the rats were given 338 g. of the food, 230 cc. of milk and 6·8 g.



Curve 6. Synthetic diet (milk salts) + milk + cholesterol 2 % (Set 8). Average weight curve.

cholesterol. As in the previous cases the food left over was carefully dried and weighed and it was found that the rats had eaten approximately 200·7 g. food and 4 g. of cholesterol. The total cholesterol in the 230 cc. of milk added was about 0·048 g. since milk contains 4 % of fat, and the fat 0·5 % of cholesterol.

One rat which met with an accident on the 15th day was killed at once. At the end of the period of dieting the remaining three rats weighed 80·3 g., corresponding to an average weight of 26·7 g. The increase in weight per rat was therefore 7·2 g. during the period of 23 days, which is less than half the average increase of the previous set.

The rats were killed on July 22. Total cholesterol found 0·4012 g., corresponding to 0·134 g. per rat, a figure approximating to that found for set (13), viz. 0·1104 g. per rat. However, growth was much slower on this synthetic diet with milk and cholesterol than on the partly extracted food with cholesterol alone added (set 13). As the total cholesterol found was

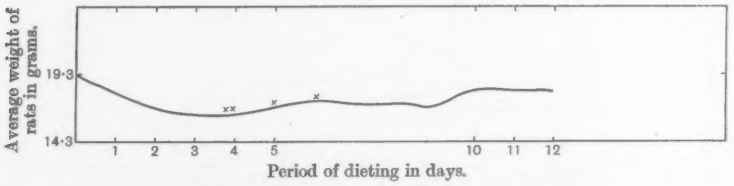
only 0·4012 g. for the three rats and 4 g. had been eaten in the food, it is clear that only about one-tenth of that ingested is stored up.

Curve 6 represents the average weights of these rats plotted against days.

Set (9). This set consisted of six rats which were fed with the synthetic diet used in set (4) to which 2 % of cholesterol was added. It has been seen that in the case of sets (4) and (5) no increase either of growth or of cholesterol content took place on a pure synthetic diet alone, and one wished to see what effect the addition of cholesterol would have on each of these factors. The weight of the six rats at the commencement of the period of dieting was 115·5 g., being an average of 19·3 g. Two rats died on the 4th day, one on the 5th and one on the 6th, but no nibbling whatever occurred as the bodies were removed in time to prevent it. The remaining two rats were killed on the 13th day and analysed. Their weight was then 36 g., being an average of 18 g. No increase in weight had therefore taken place. During the period of dieting the rats were given 116 g. of food, 76 of which were eaten including 1·52 g. of cholesterol. The two rats were killed on the 13th day and analysed. Total cholesterol content for the two rats 0·148 g.; or 0·074 g. per rat.

Now up to the end of the 6th day 35 g. of food had been eaten containing 0·71 g. of cholesterol. Thus the remaining 1·52 — 0·71 or 0·81 g. of cholesterol was eaten by the two rats which were analysed. The amount of cholesterol to be accounted for in their bodies is obviously 2(0·074 — 0·0359) = 0·0762 g. taking 0·0359 as standard, and this is quite covered by the quantity of cholesterol ingested. It will be noted that only about one-tenth of the cholesterol ingested is stored, the remainder being excreted in the faeces.

Curve 7 shows the average weights of the rats during the experiment.



Curve 7. Synthetic diet + cholesterol (Set 9). Average weight curve.

Set (10). This set consisted of four rats whose diet was the same as in the last set but with the following modifications:

(1) The fat employed was manufactured by dissolving glycerol in dry pyridine and adding palmitic chloride. This was allowed to stand over

night and then poured into water and filtered through a filter pump. Any excess of acid chloride was dissolved out by a dilute solution of sodium carbonate and the residual fat carefully dried.

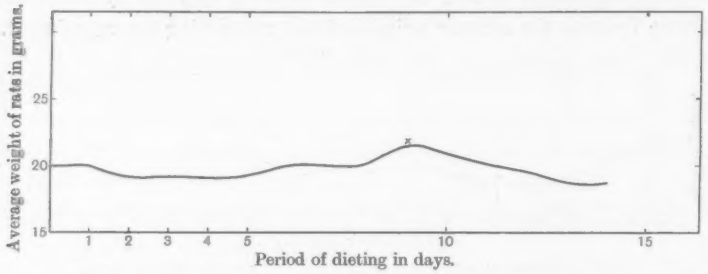
(2) 0·5 % of pure lecithin prepared from bulls' testicles was added to the food, being carefully rubbed in so as to be uniformly distributed.

(3) Half of the pure protein ordinarily employed was replaced by protein obtained from bulls' testicles which was quite cholesterol free (*vide* set 6).

The weight of the four rats at the beginning of the experiment was 81 g., being an average of 20·25 g., and at the end three rats remained whose total weight was 55 g., being an average of 18·3 g., one rat dying on the last day. It was considered that the unpalatability of the synthetic food might be mitigated by flavouring it with meat extract; this was done all through this particular experiment which lasted 15 days. The average weight nevertheless went down, neither the lemco added nor the lecithin seeming to have any pronounced effect on growth or even sufficing to sustain the original weight. During the period of dieting of 15 days the rats were given 144 g. of food and 2·88 g. of cholesterol; 101·3 g. of food were eaten; the cholesterol intake thus being 2 g.

The actual weight of material analysed was 52·6 g. Total cholesterol content 0·2654 g.; or 0·0883 g. per rat. The quantity of cholesterol which the three rats have put on is $3(0{\cdot}0883 - 0{\cdot}0359) = 0{\cdot}572$ g. These rats ingested in their food about 2 g. of cholesterol. All found is therefore accounted for by that contained in the food.

Curve 8 represents the average growth during the course of the experiment.



Curve 8. Synthetic + cholesterol (artificial fat + lecithin and lemco) (Set 10). Average weight curve.

The results so far obtained do not indicate that cholesterol itself has any appreciable influence on the growth of an animal; all that is shown is that when an animal is fed on an artificial diet which does not promote growth

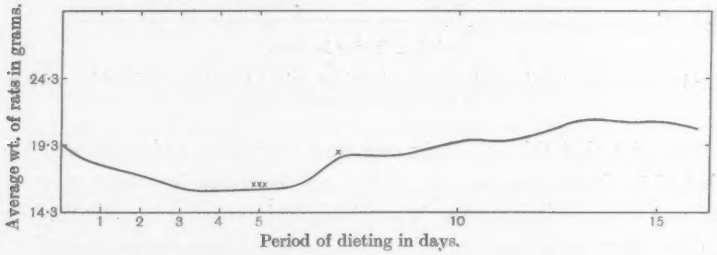
and to which cholesterol is added, no increase of growth occurs, but that part of the added cholesterol is stored bringing the total cholesterol content of an individual rat up to or above that found in the body of a rat fed on an ordinary diet [*vide* set (3)].

Whether the esters of cholesterol have any influence in this direction is a question which has been investigated by adding them to the synthetic diet instead of cholesterol.

Set (11). This set consisted of six rats fed on an ordinary synthetic diet with the addition of 2 % of cholesterol esters. The ester employed was palmitic ester which contains 0·6 of its weight of cholesterol; this is equivalent then to adding 1·2 % of cholesterol.

The weight of the six rats, at the beginning of the experiment, was 116 g., being an average of 19·3 g. per rat. On the 6th day three rats died and one on the 7th day; the two remaining rats remained alive for 16 days in good health when they weighed 43·5 g., being an average of 21·75 g. No lemco was added to the food, and no growth took place. After the four rats had died the remaining two were given 70 g. of food of which 50 were eaten, and the amount which they ate during the previous six days was calculated at 16 g. This represents 66 g. of food containing 1·2 % of cholesterol or 0·792 g. of cholesterol. These two rats were killed on the 16th day when they weighed 43·5 g., or an average of 21·75 g. Total cholesterol content 0·1496 g., or 0·075 g. per rat, representing an increase for the two rats of $2(0{\cdot}075 - 0{\cdot}0359) = 0{\cdot}08$ g.

We have seen that 0·792 g. of cholesterol was ingested so that about one-tenth of this quantity has been stored in the bodies of these two rats.



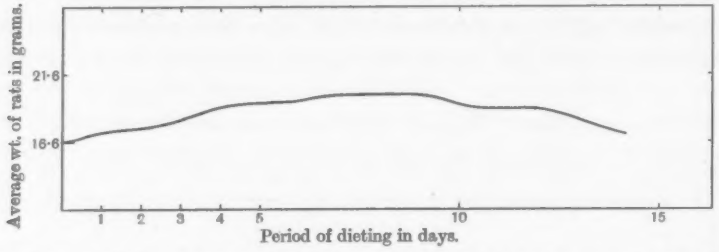
Curve 9. Synthetic + cholesterol esters (Set 11). Average weight curve.

Curve 9 gives the average weight of these rats during the course of the experiment.

Set (12). This set, consisting of six rats, was fed on exactly the same diet

as the last with the addition of 0·5 % lecithin; the fat used was synthesised as in set (10) and half the protein was replaced by the pure protein obtained from bulls' testicles [see set (6)]. On June 24, when the dieting was commenced the six rats weighed 99·5 g., being an average of 16·6 g. For the first five days lemco was used to flavour the diet, at the end of which time the total weight had gone up to 116 g. On the 6th, 7th, 8th and 9th days the lemco was omitted, by which time the total weight was 120·5 g. Lemco was now again added to the diet, but the total weight steadily declined till the 15th day, when it was 105 g. and two rats died. All through the experiment the rats had been quite well, but now began to show signs of diminished vitality; it was therefore decided to analyse them at this point when their average weight, 17 g., was practically what it was at the beginning of the experiment, viz. 16·6 g. The total amount of material analysed was 100 g. Total cholesterol content, 0·416 g., or 0·069 g. per rat. During the 15 days the six rats were given 180 g. of food of which 157 were eaten. This means that about 1·8 g. of cholesterol were also ingested. The total cholesterol put on by the six rats is $6(0{\cdot}069 - 0{\cdot}0359) = 0{\cdot}1986$ g., that is to say about one-tenth of that ingested, as was the case in the last set.

Curve 10 gives the average weight during the course of the experiment.



Curve 10. Synthetic + cholesterol esters (artificial fat, mixed proteins, 5 % lecithin, and lemco (Set 12). Average weight curve.

We notice that this set of six rats were kept alive and quite well for a period of 15 days when two died. The rats increased in weight about the middle of the experiment when it was thought interesting to omit the lemco from the diet; after four days from the omission they began to decline in weight, and the re-addition of lemco to the food failed to check the decline which progressed steadily to the end.

It has been an endeavour in these experiments to keep the rats in health for a long period on synthetic diet either with or without cholesterol and to

note the cholesterol content at the end, but so far complete success has not been obtained.

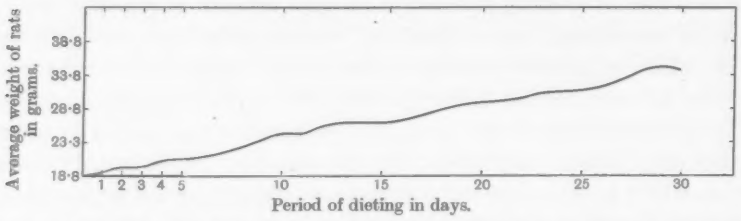
The results obtained from those sets of rats which were fed on a synthetic diet to which cholesterol and cholesterol esters were added do not go to show that these substances have any effect on the growth of the rats. The inability of a synthetic diet to enable growth processes to go on is evidently not due to the absence of cholesterol.

It seemed interesting, however, to see what would be the result of feeding rats on an ordinary diet of bread and oatmeal which had been extracted with ether, or to which cholesterol was added. Should we find the same results as were obtained in the last four sets or not? Unfortunately, the food material used was not thoroughly extracted as was proved by subsequent examination. 50 g. of the food were heated on a water bath for several hours with a 10 % alkali solution, after which the mixture was made acid and again heated for several hours. The mass was then analysed for phytosterol by the modification of Windaus' method. In the 50 g. taken was found 0·0076 g. of phytosterol, that is 0·0152 %.

From 50 g. of similar food, viz. bread and oatmeal, which had been extracted by alcohol, 0·0074 g. of phytosterol was obtained, i.e. 0·0148 %.

Set (13). This set was fed on bread and oatmeal which had been extracted with ether together with the addition of 2 % cholesterol in the daily ration. We have seen in set (5) how the rats behaved on a fat free diet, and I wished to see what effect the addition of cholesterol would have on the growth and condition of the rats. A litter of 12 rats was born on May 30, 1913. Four of these were put on the special diet on June 30 and during the period of dieting of 31 days, were given 278 g. of bread and 276 g. of oatmeal and 11 g. of cholesterol; 428 g. of bread and oatmeal were eaten and 8·5 g. of cholesterol by the four rats, none of which died. The bread and oatmeal were weighed dry, and, with the cholesterol added, were made into a paste with water of such consistency that the rats would eat it freely. The weight of the four rats at the beginning of the experiment was 75·2 g., representing an average of 18·8 g. per rat. The rats readily ate the mixture and were quite healthy throughout the period of 31 days. At the end of this period their total weight was 138·8 g., corresponding to an average of 34·7 g. per rat. Each rat had therefore gained in weight practically 16 g. Curve 11 represents the average growth during the experiment. There were no deaths and all the rats remained quite healthy. The rats were killed on July 30; the weight of the actual tissue analysed being 134·5 g. Total cholesterol 0·4544 g.,

corresponding to 0·1104 g. of cholesterol per rat. It will thus be seen that each rat has put on roughly about 0·0748 g. of cholesterol, again taking 0·0359 from set (1) as our standard. As each rat ate during the course of the experiment about 2 g. of the pure cholesterol given, it will be seen that only a small proportion of that ingested is stored. It is as well to note that the 428 g. of food eaten would contain in all 0·064 g. of sterol which had not been extracted, but this will in no way alter our results.



Curve 11. Ether extracted bread and oatmeal + 2 % cholesterol (Set 13). Average weight curve.

It would seem, in view of the results obtained from sets (9), (10), (11) and (12) which were fed on synthetic diets to which cholesterol and cholesterol esters were given and in which no growth occurred, that the growth in this case is due rather to the fact that the food was imperfectly extracted than to the fact that cholesterol was added.

The results of the experiments detailed above are gathered together in Tables I and II (pp. 81, 82).

Summary of Results.

1. The rats which were just beginning to feed themselves contained in their bodies a normal standard quantity of cholesterol, viz. 0·0359 and 0·038 g. for sets (1) and (2) respectively. The total percentage is 0·1467.

2. After being fed on an ordinary diet of bread and oatmeal for three weeks, the total quantity of cholesterol per rat goes up to 0·071 g., the percentage being 0·2.

3. When the rats are fed on a pure synthetic diet containing no cholesterol, no growth occurs, nor is there any increase in the cholesterol content of the organism over a period of 16 days, and correspondingly there is no loss (sets 4 and 5).

4. The replacement of half of the pure caseinogen used in the synthetic diet by cholesterol-free protein obtained from bulls' testicles, together with

the addition of lecithin, also causes no increase either in the growth or the cholesterol content of the rats (set 6).

5. When rats were fed on the ordinary synthetic diet, with the addition of considerable quantities of milk, viz. 20 cc. *per diem*, they increased their weight by 66 % in 23 days. The cholesterol in the milk was eagerly picked out by the organism and stored, and the quantity found could be accounted for by that contained in the milk. There was no evidence of any synthesis (set 7).

6. When half the above quantity of milk was given and in addition 2 % of cholesterol, growth was considerably less during the same period of 23 days. About one-tenth of the cholesterol ingested was stored, bringing the total quantity per rat up to 0·1340 g. and the total percentage to 0·5236 (set 8).

7. The addition of cholesterol and cholesterol esters to a synthetic diet, together with lecithin and lemco, did not in any way enable normal growth to proceed. At the end of 15 or 16 days the average weight of the rats fed on a synthetic diet with cholesterol added had slightly decreased, and in the sets which had cholesterol esters added the increase per rat was less than 1 g. during 16 days. In all of these cases the rats stored in their bodies about one-tenth of the total cholesterol eaten with the food (sets 9, 10, 11 and 12).

8. When a diet of bread and oatmeal which had been imperfectly extracted with ether was given together with 2 % of cholesterol, slight growth occurred and the total quantity of cholesterol in the rat was found to be about three times that of set (1). (Set 13.)

General Conclusions.

A pure synthetic dietary is insufficient to enable "growth processes" to go on in young rats, in other words the rats are starved on such a diet and neither cholesterol, cholesterol esters nor lecithin appears to supply the deficiency. Lemco, however, appears to have a beneficial effect in some cases, rats fed on a diet with this addition showing no eagerness to nibble their dead comrades.

There is no evidence that the living organism can manufacture cholesterol, but on the other hand it is a substance which is strictly conserved and readily picked out from a diet in which it is present in only small quantities. When, however, it is present in large quantities in the food then the percentage in the body goes up considerably but only a small quantity of that ingested is actually stored up.

I take this opportunity of thanking the Grants Committee of the Dixon Fund for help in carrying out this work, and also Mr J. A. Gardner for advice during the progress of the work.

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X. THE SPECIFICITY OF CASEINOGENS. A COMPARATIVE STUDY OF THE CASEINOGENS OF THE COW AND THE SHEEP.

By HAROLD WARD DUDLEY
AND HERBERT ERNEST WOODMAN.

From the Animal Nutrition Research Institution, The University of Leeds.

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For many years the question as to whether the caseinogens derived from different species of mammals are one and the same chemical substance has remained undecided.

The general physical and chemical characteristics of the caseinogens precipitated from various milks by dilute acid are so similar that no satisfactory conclusions can be drawn regarding the question of their chemical identity, although some earlier investigators have either supported the hypothesis of chemical identity on the strength of the general similarity of the substances or opposed it on account of observations of slight differences.

Abderhalden and Schittenhelm [1906] compared the amounts of amino-acids contained in the caseinogens of cow's and goat's milk with the result that a striking similarity was demonstrated and the assertion of differences in the quantitative amino-acid composition of the two proteins was not possible.

Elementary analyses of caseinogens coincide so closely that they have been adduced as evidence in favour of the view that the caseinogens of various mammals are identical. The comparison of cow's and sheep's caseinogens, with which this paper is especially concerned, serves to show how closely the analyses agree.

Cow	C 52·96 %	H 7·05 %	N 15·65 %	S 0·758 %	P 0·847 % [Hammarsten, 1883, 1885]
Sheep ..	C 52·92	H 7·05	N 15·60	S 0·771	P 0·809 [Tangl, 1908]

Nevertheless it has long been suspected that as a matter of fact there may be differences in the structure of various caseinogens, not necessarily in respect to the relative amounts of amino-acids present, but possibly in the actual arrangement and order of these acids within the molecules. This is a reasonable assumption on biological grounds, and biological experiments—the formation of specific precipitins on injection into the blood-stream of pure caseinogen preparations—have given support to this view.

In this paper, however, we bring forward for the first time definite chemical evidence in support of the general assumption of specificity in caseinogens, and proof in particular of a difference in intramolecular structure between cow's and sheep's caseinogens. We have been enabled to do this by a comparative study of the amino-acids derived from the "racemised" caseinogens. If caseinogen be dissolved in dilute alkali and the solution allowed to stand at 37° for about three weeks the value of the optical rotation falls to a practically constant value. The protein is then said to be "racemised" and the amino-acids obtained on subsequent hydrolysis are found to be in some cases optically inactive, in others partially active and in others fully active. Dakin [1912] has shown that in all probability the optical properties of the amino-acids depend upon their positions in the peptide chains. According to his view amino-acids whose carboxyl groups are free and which therefore may occupy terminal positions at the ends of peptide chains, are incapable of racemisation, whilst those whose carboxyl groups are united in actual peptide linkages, and are therefore probably situated within the chains, may undergo racemisation. The optical activity or inactivity of the amino-acids among the hydrolytic products of a "racemised" protein will therefore give some clue as to the original positions of these acids in the protein molecule, although the full interpretation of the significance of these findings is not yet possible.

Accordingly we "racemised" a quantity of sheep's caseinogen with dilute alkali under precisely the same conditions as those employed by Dakin and Dudley [1913] in an investigation of the racemisation of cow's caseinogen. By a comparison of the optical properties of the corresponding amino-acids derived from "racemised" cow's and sheep's caseinogens we hoped possibly to be able to detect structural differences relating to the positions of the respective amino-acids in the molecules of the two proteins. In this we were successful; the following table compares the optical properties of the amino-acids from racemised cow's and sheep's caseinogens respectively:

	Racemised cow's caseinogen	Racemised sheep's caseinogen
Alanine	inactive (and d?)	inactive (and d?)
Valine	*d.* and inactive	*d.* and inactive
Leucine	*l.* and inactive	*l.* and inactive
Tyrosine	inactive	*l.*
Phenylalanine	inactive	inactive
Proline	*l.*	*l.*
Aspartic acid	inactive	inactive
Glutaminic acid	inactive	inactive and *d.*
Arginine	inactive	inactive
Histidine	inactive	inactive
Lysine	inactive	*d.*

It will be seen that, as is to be expected, most of the amino-acids from the two caseinogens display identical optical properties, indicating a general similarity in the molecular structures of the two proteins. But a sharp difference is found in the case of tyrosine. The tyrosine derived from "racemised" cow's caseinogen is completely inactive, that from "racemised" sheep's caseinogen is fully active.

Again, the lysine derived from cow's caseinogen is totally inactive, but the specimen isolated from sheep's caseinogen displayed two-thirds of the full activity. The lysine fraction was originally contaminated with some *l.* proline, which was only removed with difficulty by fractional precipitation of the phosphotungstates. Bearing in mind the possibility that traces of *l.* proline might still cling to the lysine, together with the fact that lysine is not a very stable substance, it seems probable that no racemisation of lysine took place before hydrolysis. Further, we find that a small quantity (about 15 %) of the total glutaminic acid obtained from "racemised" sheep's caseinogen is optically active, while that derived from cow's caseinogen is completely inactive.

Although the significance of these differences cannot yet be fully elucidated, they may be taken at least as direct proof that cow's and sheep's caseinogens are two distinct proteins. Their general structure is undoubtedly very similar and might even be, with regard to amino-acid content, quantitatively practically identical, but small definite differences in the arrangement of the amino-acids within the molecules of the two substances must occur.

We incline to the view that each species of mammal may synthesise its own specific caseinogen, and therefore that every caseinogen is a distinct member of a class of very similar proteins.

One point of difference in the comparisons of the amino-acids from the two caseinogens lies in the fact that in the investigation of cow's caseinogen the "racemised" protein was precipitated from the alkaline solution after

"racemisation" and hydrolysed separately (except in the isolation of tyrosine), whereas the alkaline solution of sheep's caseinogen was hydrolysed as it stood, thus including the products of the hydrolysis which proceeds simultaneously with "racemisation." We do not, however, consider that this procedure vitiates our present results because it has been shown [Dakin and Dudley, 1913] that the amino-acids from "racemised" caseose, the main hydrolytic product during the process of "racemisation," are identical with those of "racemised" caseinogen, and that the quantity of free amino-acids formed under the experimental conditions of "racemisation" is very small.

Fortunately our most definite finding, the difference between the two specimens of tyrosine, is in any case not affected by this possible criticism, since in the case of both caseinogens the entire alkaline solutions were hydrolysed as they stood after digestion, and worked up in exactly the same way.

We propose to extend these investigations to the study of the relationships between other proteins.

In conclusion we desire to thank Dr H. D. Dakin for permission to apply the method of protein racemisation to problems of this nature.

Experimental.

Preparation of sheep's caseinogen.

The caseinogen was precipitated from the "separated" milk by means of dilute acetic acid. The best conditions for precipitation were found to be as follows.

A 0·06 % (vol.) solution of acetic acid was prepared in a large glass vessel and for each litre of this solution 50 cc. of milk were added with vigorous stirring. The caseinogen, which flocked out almost immediately, was allowed to settle and the clear liquid was siphoned off. The caseinogen precipitate was washed with distilled water until free from soluble proteins. If tap water is used the preparation becomes discoloured. After filtering on a large Buchner funnel the caseinogen was ground up in a mortar with an alcohol-ether (4 : 6) mixture, filtered, ground up again with ether, filtered and dried in vacuo over sulphuric acid.

Racemisation of sheep's caseinogen.

This was carried out exactly as in the case of cow's caseinogen [Dakin and Dudley, 1913], 10 % solutions of the caseinogen in $n/2$ sodium hydroxide being digested at 37° for 20–24 days. The fall in rotation and the amount of rotation were of a similar order to those recorded for cow's caseinogen.

Isolation of Amino-acids.

Tyrosine. 50 g. sheep's caseinogen were racemised. The alkaline solution was then hydrolysed with hydrochloric acid, the solution filtered and evaporated in vacuo at 45°. The residue was then taken up in water, boiled with animal charcoal and filtered. The solution, rendered slightly alkaline with ammonia, was concentrated and tyrosine was allowed to crystallise. The crude tyrosine was filtered off, washed with small quantities of hot glacial acetic acid, and then dissolved in hydrochloric acid. The solution, after treatment with animal charcoal, was neutralised with ammonia and on cooling tyrosine crystallised in characteristic form. It was found to be fully *laevo*-rotatory. It was compared with a specimen of pure *l.* tyrosine prepared by the pancreatic digestion of cow's caseinogen. In 4 % HCl solution the two specimens showed identical specific rotations: − 11·60° and − 11·65°.

400 g. sheep's caseinogen were racemised in the usual way and then hydrolysed with hydrochloric acid for investigation of the amino-acids by the esterification method. Levene's modification of Fischer's method [Levene and van Slyke, 1909] was employed.

Alanine. A specimen of pure *i*-alanine was obtained by fractional crystallisation. Other fractions containing alanine and valine were recovered which possessed a *dextro*-rotation. They may have been mixtures of *d.* alanine and *d.* valine, or possibly of *i.* alanine and *d.* valine.

Valine was isolated in mixtures of *i.* and *d.* valine containing over 50 % of the active form. The *dextro*-variety was also found in leucine-valine mixtures and in valine-alanine fractions.

Leucine was obtained in the pure state almost inactive, and fractions of *l.* leucine containing only very small amounts of *d.* valine were also separated.

Phenylalanine was readily obtained in the pure state and was found to be totally inactive.

Proline was isolated and displayed the partial racemisation which always occurs as a result of the process of separation. Its activity was such as to render it probable that no racemisation had occurred before hydrolysis.

Aspartic acid was isolated in the inactive form. None of the active variety could be detected.

Glutaminic acid was obtained in large quantity and proved to be a mixture of the inactive acid with about 15 % of the *dextro*-form.

Arginine, histidine and lysine were isolated by the method of Kossel and

Kutscher. 150 g. sheep's caseinogen were racemised for this purpose. Arginine and histidine were both inactive. The lysine fraction displayed a definite *laevo*-rotation when first isolated. By fractional precipitation of the phosphotungstates a specimen of *l.* proline was removed and found to be responsible for the original *l.* rotation. The lysine, examined as the dihydrochloride, now displayed a specific rotation of $+9{\cdot}4°$. It was identified by the preparation of the characteristic picrate.

Summary.

By comparing the optical properties of the amino-acids derived from "racemised" cow's and sheep's caseinogens it is shown that the intramolecular arrangement of the amino-acids in these two proteins is not identical, and it is considered probable that each milk-producing species may synthesise its own specific caseinogen.

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XI. LIGNOCERIC ACID FROM "CARNAUBON."

By OTTO ROSENHEIM and HUGH MACLEAN.

From the Physiological Laboratory, King's College, London, and the Pathological Chemical Laboratory, St Thomas's Hospital, London.

(*Received February 8th, 1915.*)

Nearly ten years ago Dunham [1905] discovered that ox kidneys contained a substance resembling the so-called "protagon" which Liebreich had found in brain. On comparing the two substances prepared by identical methods from brain and from kidneys (extraction of the fresh tissue with 85 % alcohol at 45°, or with boiling alcohol after the coagulation of the proteins by 5 % sodium sulphate solution), he found that "the substance from the kidney contains distinctly more nitrogen and phosphorus than that from the brain," but that "the cleavage products show that all of these substances belong to the same group."

Later on, however, Dunham [1908] succeeded in isolating from the kidney-"protagon"[1] an acid of the composition $C_{24}H_{48}O_2$ which he considered identical with carnaubic acid. As this fatty acid of vegetable origin had not been previously found in animal tissues, Dunham and Jacobson [1910] gave the name "carnaubon" to this "protagon"-like lipoid of the kidney. From their hydrolysis experiments they came to the conclusion that "carnaubon" represented a triamino-monophosphatide in which an amino-galactose is connected with three fatty acids (carnaubic, stearic and palmitic acid) and with phosphoric acid, whilst the latter is still further connected with two choline groups.

Maclean [1912], in his investigation of the phosphatides of the kidney, obtained a similar lipoid from horse kidney, but he found that the substance contained a water-soluble nitrogenous impurity and that after its removal

[1] Under this name the substance has already passed into the literature. [See Oppenheimer, 1909.]

the nitrogen : phosphorus ratio had fallen from 3 : 1 to 2 : 1. The substance resembled the diamino-phosphatide sphingomyelin in its general properties, but it still furnished a reducing sugar on hydrolysis.

In the course of some unpublished experiments on the products of hydrolysis of brain-"protagon," Rosenheim noticed that the mixture of fatty acids contained one which closely resembled the carnaubic acid described by Dunham as a typical constituent of "carnaubon." Rosenheim found further [1913, 1, 2] that of the constituents of "protagon" the galactoside kerasin yielded this acid (which he identified with lignoceric acid) as its characteristic fatty acid, whilst the phosphatide sphingomyelin also contained it in smaller amounts. Similar results were obtained simultaneously by Levene [1913] and by Thierfelder [1913], who, however, called the acid "kerasinic acid."

In the light of this experience it seemed of interest to reinvestigate the fatty acids of "carnaubon." We prepared therefore this lipoid from ox kidneys as well as from horse kidneys according to Dunham and Jacobson's directions and hydrolysed the products by alcoholic sulphuric acid. We obtained in confirmation of Dunham's results a fatty acid of the composition $C_{24}H_{48}O_2$, but we were able definitely to identify this acid with lignoceric acid.

There seems therefore no further justification for the retention of the name "carnaubon" for the "protagon"-like lipoid of the kidney[1].

We were unable to find palmitic and stearic acid amongst the products of hydrolysis of "carnaubon," but isolated instead a fatty acid of a higher melting point (94°); this was characterised by its acetyl-derivative as the hydroxy-acid, phrenosinic acid ($C_{25}H_{50}O_3$), which is the fatty acid of the galactoside phrenosin[2].

Moreover, we found that the basic products of hydrolysis contained not only choline, which Dunham and Jacobson considered as the only base present, but also an ether-soluble base identical with sphingosine, a base also obtained on hydrolysis of brain-"protagon" [Rosenheim and Tebb, 1907].

These facts cannot be made to harmonise with the views of Dunham and Jacobson as to the constitution of "carnaubon." If we consider in conjunction with the above results the fact that the elementary composition of

[1] It may be pointed out in this connection that the uniformity of "carnaubic" acid itself has recently been made doubtful. [Röhmann, 1905, Matthes and Sanders, 1908, Lewkowitsch, 1913.]

[2] Palmitic and stearic acid were not satisfactorily identified by Dunham and Jacobson (no melting points are given) and their combustion results are in fair agreement with those required by phrenosinic acid, containing an admixture of lignoceric acid.

different preparations of "carnaubon" is extremely variable[1], we come to the conclusion that the kidney-"protagon" represents a variable mixture of lipoids, mainly of galactosides (cerebrosides) and sphingomyelin. In distinction from brain-"protagon" it seems that sphingomyelin preponderates in kidney-"protagon."

Experimental.

"Carnaubon" was prepared from ox kidneys according to Dunham and Jacobson's directions. From 8 kg. fresh ox kidneys were obtained 2·93 g. of twice recrystallised product, which after treatment with water and two further recrystallisations yielded 1·0 g. of carnaubon. In another preparation horse kidneys were employed, about 2 kg. being worked up at a time until 2·5 g. of "pure" carnaubon were available.

Ethyl ester of lignoceric acid.

0·7 g. of carnaubon (ox) was hydrolysed by boiling for six hours under a reflux condenser with 35 cc. absolute alcohol containing 8 % conc. sulphuric acid. The ester crystallised out on cooling in glistening white crystals, which were filtered off on hardened filter paper, washed with cold alcohol and dried in vacuo. The crystals could be easily separated from the paper after drying as a felted silky sheet, which weighed 0·11 g. The ester was twice recrystallised from acetone and finally dried in vacuo at 35°.

It melted at 56° and solidified at 54°. Mixed with lignoceric acid ester from wood paraffin[2] it showed exactly the same melting and solidifying point.

The quantity of the ester available was sufficient for two micro-combustions.

(1) 13·050 mg.; 37·600 mg. CO_2; 15·175 mg. H_2O.
(2) 8·465 mg.; 24·275 mg. CO_2; 9·840 mg. H_2O.

	(1)	(2)	Calc. for $C_{22}H_{47}COOC_2H_5$
C	78·58 %	78·21 %	78·71 %
H	13·01 %	13·01 %	13·22 %

2 g. of carnaubon (horse) were hydrolysed by boiling with 100 cc. absolute alcohol containing 5 % conc. sulphuric acid as before. 0·47 g. of the ester was obtained, which was recrystallised from acetone as above. It showed a

[1] The elementary composition of "carnaubon" obtained by Dunham [1905], Dunham and Jacobson [1910] and Maclean [1912] shows the following variations:

C %	H %	P %	N %	S %
65·61–68·19	11·00–12·37	2·02–3·44	2·55–3·26	0–0·82

[2] Prof. Hell, the discoverer of lignoceric acid [1880], kindly put at Dr Rosenheim's disposal some years ago samples of his original lignoceric acid and its ethyl ester, for which we wish to take this further opportunity of thanking him.

melting point of 56·1°–56·4° and solidified at 53·6°. A mixture with Hell's ethyl ester of lignoceric acid melted at the same temperature.

Molecular weight estimation. 0·3835 g. was saponified by boiling for three hours with 16·04 cc. 0·1 N alcoholic potash. The excess of potash was then titrated back by means of 0·1 N HCl. 9·71 cc. of 0·1 N KOH had been used up. M.W. = 395. Calc. for $C_{23}H_{47}COOC_2H_5$: 396.

Lignoceric acid.

The potassium salt of the acid was prepared from the ester by saponification with alcoholic potash. The salt was well washed with alcohol and acetone, dried in vacuo and the acid set free by dilute sulphuric acid. The free fatty acid was repeatedly washed with water by fusing its watery suspension on the water-bath. It was then extracted with ether, the ether solution washed with water in a separating funnel, filtered through dry filter paper and evaporated. The white crystalline residue of the free acid was recrystallised from a mixture of acetone and petroleum ether. It was obtained in silky white crystals, which assumed a wax-like consistency when compressed in an agate mortar.

It melted sharply at 81° and solidified at 74·2°–73·5°. Samples were mixed with Hell's lignoceric acid, further with lignoceric acid prepared from arachis oil [Kreiling, 1888] and with lignoceric acid from kerasin [Rosenheim, 1913, 1, 2].

The melting points of the mixtures are given in the following table.

		Melts at	Solidifies at
Acid from "carnaubon" mixed with	Lignoceric acid (Hell)	81·1°	74·3–73·4°
	Lignoceric acid from arachis oil	81·0°	74·5–73·5°
	Lignoceric acid from kerasin	81·0°	74·4–73·5°

Molecular weight estimation. 0·2192 g. of the acid, dissolved in benzene and petrol ether, was titrated with 0·1 N alcoholic potash (phenolphthalein as indicator) and required 6·00 cc. 0·1 N KOH.

M.W. = 365. Calc. for $C_{24}H_{48}O_2$: 368.

From the potassium salt obtained in the above molecular weight estimation the acid was again set free as before and after a further recrystallisation showed the same melting point (81·1°) as before.

Lead salt.

0·1 g. of the acid, twice purified by means of the potassium salt, was dissolved in methyl alcohol and precipitated with a solution of lead acetate in methyl alcohol. A drop of dilute methyl alcoholic ammonia was added to complete the precipitation. The voluminous white lead salt was filtered, washed with hot acetone and dried in vacuo. It was obtained as a white heavy powder which melted at 117°. Hell and Hermanns [1880] gave the melting point of their lead lignocerate as 117°.

0·1289 g. gave 0·0412 g. lead sulphate

	Calc. for $(C_{24}H_{47}O_2)_2$ Pb
Pb: 21·83 %	21·99 %

For the purpose of isolating the other products of hydrolysis, the original alcoholic solution (after the filtration of the ester of lignoceric acid) was diluted with water and the alcohol evaporated on the water-bath. An oily semi-solid substance gradually collected on the surface, and solidified on cooling. This substance was found to consist of a mixture of phrenosinic acid (and its ester) and sphingosine sulphate, which were separated by means of ether, in which the sulphate of the base is insoluble.

Phrenosinic acid[1]. After evaporation of the alcohol the opaque watery fluid was extracted with ether. The ether residue was dissolved in alcohol and neutralised with alcoholic potash. An insoluble potassium salt settled down, from which the fatty acid was set free by dilute sulphuric acid. The free acid was taken up in ether and afterwards recrystallised twice from acetone. It crystallised from alcohol in the characteristic "mamillary" masses which have already been described by Thudichum and later by Thierfelder. The substance melted at 92° and after two recrystallisations at 93°–94°. Levene and Jacobs [1912] have shown that phrenosinic acid exists in two optically isomeric forms, of which the inactive one melts at 82°–85° (Thudichum's neurostearic acid) and the dextro-rotatory form at 106°–108° (Thierfelder's cerebronic acid), whilst a mixture of the two melts at 92°–95°. The acid obtained by us from "carnaubon" is evidently a mixture of the two isomeric forms. It is interesting to note that Grey [1913] also isolated a phrenosinic (cerebronic) acid of the melting point 91° from the mixed fatty acids of brain.

Acetyl-phrenosinic acid. The free acid was boiled for two hours with an excess of acetic anhydride. After cooling, the crystalline acetyl product was

[1] Phrenosinic acid = Thudichum's "neurostearic" acid = Thierfelder's "cerebronic" acid. [See O. Rosenheim, 1914.]

filtered, washed with alcohol and dried in vacuo over soda lime. It was recrystallised from petrol ether and melted at 64°–65°. It solidified at 62°–63°. According to Levene and West [1913] the acetyl derivative of the inactive phrenosinic acid "melts at 55·5°–56° and solidifies at 53°–54°. The active form would melt higher."

Sphingosine. After the extraction of the phrenosinic acid by ether, the somewhat gelatinous precipitate of sphingosine sulphate left in the aqueous hydrolysis fluid was filtered and washed. The substance was dissolved in alcohol and the base set free by means of alcoholic potash. After dilution with water, it was extracted with ether and remained after removal of the ether as a partly crystalline mass. The free base was converted into the sulphate by neutralising its alcoholic solution with alcoholic sulphuric acid. The insoluble sulphate was filtered off and recrystallised twice from alcohol. It crystallised in needles arranged in rosettes and was finally obtained as a white crystalline powder. On heating it gradually decomposes and melts at 243°–244°, effervescing at 250°. Thierfelder [1904] states that it decomposes with effervescence between 240°–250°. The substance gives with sulphuric acid and sucrose a typical Pettenkofer's reaction and yields nitrogen with nitrous acid. A quantitative estimation in van Slyke's micro-apparatus was unfortunately lost owing to unexpected excessive frothing.

The final aqueous hydrolysis fluid gave Molisch's test and contained a reducing sugar (galactose?) and phosphoric acid. The presence of choline was demonstrated by the periodide test.

We are at present engaged with the preparation of larger quantities of the material, in order to separate the galactosides from sphingomyelin by the pyridine method [Rosenheim, 1914] and to investigate further their cleavage products.

The expenses of this research have been in part defrayed from a grant to Dr O. Rosenheim from the Government Grant Committee of the Royal Society.

Summary.

1. The fatty acid $C_{24}H_{48}O_2$ obtainable on hydrolysis of "carnaubon" is not carnaubic acid, but is identical with lignoceric acid.

2. Phrenosinic acid ($C_{25}H_{50}O_3$) and the base sphingosine ($C_{17}H_{35}NO_2$) were isolated as hitherto unrecognised cleavage products of "carnaubon."

3. There is no justification for the retention of the name "carnaubon" for the lipoid mixture which can be obtained from kidneys by extraction with alcohol.

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XII. RESEARCHES ON THE INHIBITION PRODUCED BY CERTAIN SERA ON THE COAGULATING POWER OF RENNET.

By AAGE CHRISTIAN THAYSEN.

From the Lister Institute, London.

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Introduction.

Among the numerous papers of the last 20 years dealing with the reaction of rennet and its antibodies, those of Morgenroth [1899], Korschun [1902] and Hedin [1906, 1909] have been of particular interest.

In order to compare the effects of enzymes and toxins Morgenroth immunised goats against rennet and was thus able to make a rennet antiserum, which had a strong inhibiting effect upon the activity of rennet. That this inhibiting effect was due to antibodies, formed in the antiserum during the immunisation, and not to some substance in the normal goat serum, is demonstrated by Morgenroth in the following manner. Fresh milk and milk mixed with normal goat serum require exactly the same amount of rennet for their coagulation, whereas milk mixed with the antiserum in the same proportions as milk with normal goat serum only coagulates in presence of a much greater quantity of enzyme. Before Morgenroth's investigations were carried out, Hammarsten and Rödén [1887] had shown that a similar inhibiting effect may be obtained when milk is mixed with normal horse serum. In addition to this, Briot [1900] investigated the blood serum of other animals and showed that the serum of all the species examined had a more or less distinct effect on the coagulating power of rennet. In addition Briot immunised rabbits with rennet and obtained an antiserum similar to that which Morgenroth had made in goats.

In later investigations on the reaction of rennet and its antibodies the preparation of an antiserum has, however, not been attempted, because normal horse serum has a strong inhibiting effect on rennet, which was supposed to be due to a true antibody, and this obviated the necessity of preparing one. Korschun adopts this view in his publication of 1902. His

researches, which are of considerable interest, bring him to the conclusion that normal horse serum contains two substances with inhibiting action on rennet, one having a large range of action, from 20°–37°, and the other being especially active at 37°. Korschun calls this second substance "pseudo-antilab" (pseudo-antirennet).

A more detailed account of a few of Korschun's experiments will show what led him to accept the above-mentioned theory. In order to determine the coagulating power of milk, Korschun uses the quantitative method, introduced by Morgenroth, which is undoubtedly the most reliable we have. In contradistinction to the others, this method does not indicate the coagulating power of the milk as a function of the time needed for the coagulation of a certain quantity of milk by a constant quantity of rennet, but by the minimal amount of the enzyme, which is sufficient to coagulate this quantity of milk. This method, however, cannot give reliable results, when the coagulation of the milk, that is, the transformation of the colloid caseinogen into precipitated casein is brought about in the usual way, i.e. by heating the milk-rennet complex to about 35° until the transformation has taken place. For by this heating a rather considerable amount of rennet may be destroyed without it having any effect on the caseinogen. Morgenroth's method therefore makes the transformation of the caseinogen take place at lower temperatures (0°–8°), which do not injure the rennet, so that even very small quantities may remain active for a long time. At the low temperature, however, the casein formed is not precipitated and it is therefore necessary to raise the temperature of the milk-rennet complex to about 35°, when all the caseinogen has been transformed. At 35° the precipitation takes place rapidly, often instantaneously. The transformation of caseinogen into casein proceeds only slowly at 0°–8°, and to ensure that the reaction should come to an end, the milk-rennet complex has to be kept at low temperature for 12–16 hours.

Morgenroth's quantitative method is very exact, and the same milk tested at various times always indicates the same coagulating power, which is by no means the case, when this power is determined by the time which is taken for the coagulation of a certain quantity of milk with a constant quantity of rennet.

Korschun's method of testing the power of his serum is also much more reliable than the methods previously used. In these earlier methods various quantities of serum and milk were mixed and the time noted which a certain quantity of rennet required for coagulating the mixture of milk and serum at the optimal temperature of the reaction (i.e. 35°). The inhibiting power of

the serum was then expressed by the difference between the time taken for the above-mentioned process and the time required for the coagulation of the same quantity of milk without serum by the same amount of rennet. Such a method, however, only gives quite a rough idea of what really happens, because by mixing serum and milk, both these substances act on the rennet, when this is being added to the mixture, and a part of the enzyme will therefore be adsorbed by the milk, and thus rendered incapable of acting with the serum. Hedin proved, in fact, some years later, that serum has the greatest inhibiting effect on rennet, when the serum and rennet are previously mixed, and milk added to them only when the action of the serum has come to an end. The same way of testing the action of serum on rennet is used in Korschun's experiments. Increasing amounts of rennet solution are added to a constant quantity of serum, and after this mixture has been kept for 15 minutes at 20°, 10 cc. of milk are added. The samples are kept all night (12–16 hours) in the ice room, and then moved to 35° in order to determine in which samples the caseinogen of the milk has been transformed into casein. The tube, which contains the greatest quantity of rennet, and is still liquid, indicates the limit of the inhibiting power of the serum. The degree of this inhibiting power is given by the ratio of rennet used to the smallest amount of enzyme, just sufficient to coagulate 10 cc. of the same milk without serum. For example, if 10 cc. of normal horse serum are able to neutralise the effect of 0·03 cc. of a rennet solution on 10 cc. of milk, whilst 0·0006 cc. rennet is sufficient to coagulate 10 cc. of the same milk without serum, then the inhibiting power of this serum is equal to 50.

Using the above-mentioned method Korschun examines the inhibiting power of fresh normal horse serum, of dialysed and of heated horse serum, and finds that dialysis does not decrease the inhibiting power. By heating the serum, however, its effect is lost. He shows, moreover, that a horse antiserum, made by immunising a goat against horse serum, neutralises the inhibiting effect of the normal horse serum. From these facts he concludes that the inhibiting effect of the normal horse serum is due to a true antibody.

Experimenting with horse serum Korschun observed that this serum neutralises more rennet than might be expected, when the rennet-serum complex is kept at 35° or 40° instead of at 20°, and that the increase of the inhibiting power at 37°–40° is greater, up to a certain point, the longer the rennet-serum mixture is kept at the higher temperature. At 20° on the contrary no such increase in the inhibiting faculty seems to take place. He assumes therefore that in addition to the true antibody, the normal horse

serum contains a second inhibitory substance, the pseudo-antirennet, which is only active at higher temperatures.

As the dialysed serum does not show the greater effect on rennet at higher temperatures, which is shown by normal horse serum, the pseudo-antirennet must according to Korschun be dialysable. On the other hand, a heated horse serum has a strong inhibiting effect at 37° and no effect at 20°; the pseudo-antirennet appears therefore to be highly thermostable.

Hedin's researches point in quite another direction than Korschun's. In a number of publications he discusses the inhibiting influence of different substances on the activity of trypsin and rennet. In regard to rennet he points out that the intensity of the inhibiting effect depends on the order in which rennet, serum and milk are mixed. As already mentioned, he finds that the greatest effect is obtained when rennet and serum are first mixed, and the milk added to this complex, when the action of the serum has been brought to an end. Furthermore, Hedin shows that the longer the complex of rennet and serum is kept, before the milk is added, the greater up to a certain point is the amount of rennet neutralised, and that the maximum amount of the enzyme, which the serum is capable of destroying under the conditions observed, is different for different temperatures, the serum being more active at a higher than at a lower temperature. The concentration of the serum, however, does not seem to have any influence at all on the amount of rennet destroyed, and the inhibiting effect therefore is equally intense, whether the serum used is diluted or not.

Particular interest attaches to Hedin's observation that charcoal affects the rennet in the same way as normal horse serum, thus tending to indicate that the inhibiting effect of this serum is of a less complicated nature than was supposed by Korschun, and that it may possibly be due to a single character of the serum—its adsorbing quality. The further question then arises as to whether the inhibiting effect of the artificially produced rennet antiserum is of a nature similar to that of the normal horse serum, or due to the presence of a true antibody, formed in the serum during the immunisation.

In the experiments discussed below I have examined the inhibiting effect of normal horse serum, in order if possible to obtain agreement between Korschun's and Hedin's theories, which, as already shown, differ in various respects. At the same time the effect of the antirennet of normal horse serum has been compared with the inhibiting power of the antirennet formed in a rabbit serum after an immunisation, with the view of establishing their identity or otherwise.

I. Researches on the Inhibiting Substances of Normal Horse Serum.

(a) *The true antibody of horse serum and its mode of action.*

The capacity of neutralising a maximum amount of rennet in a very short time (a few minutes) is, according to Korschun, one of the most distinct qualities of the true antirennet, found in normal horse serum. Korschun therefore determines the inhibiting effect of this antibody by mixing horse serum and rennet solution, keeping the complex at 20° for 15 minutes, and measuring the remaining amount of active rennet.

In contradistinction to the action of the true antibody, the inhibiting effect of the so-called pseudo-antirennet only takes place after some time. For the determination of the effect of this substance therefore it is necessary to keep serum and rennet in contact with each other for at least three hours.

From Korschun's work it is evident that the true antibody is fully active at 20°, whereas the pseudo-antirennet does not show any distinct inhibiting effect at this temperature. Furthermore, as the effect of the true antibody does not increase proportionally to the time during which the serum acts on the rennet, it might be expected that the normal horse serum would behave similarly at 20°.

Experiment 1. (Table I.)

In this, as in all other experiments mentioned in this paper, Morgenroth's quantitative method has been used to determine the amount of rennet not neutralised by the serum in question. 10 cc. of milk were added to the rennet-serum complex, after this had been kept for the time required at 20°. The samples were then placed at a low temperature (0°) over night, and the following morning heated in a water-bath to 35°. The caseinogen of the milk was regarded as unaltered in those samples which remained liquid after half an hour at 35°, i.e. in such samples there had not been rennet enough left to transform the caseinogen into casein.

The minimal quantity of rennet solution required for coagulating 10 cc. of the milk used was 0·0015 cc.

The rennet solution in question was a standard solution, made at The Swiss Institute for Dairy Bacteriology, Liebefeld near Berne, according to Morgenroth's instructions. In cases where the amount of rennet used was less than 0·015 cc. the standard solution was diluted (1–10) with saline solution.

The milk used for the experiments was a mixed milk saturated with chloroform, and curdling as mentioned above by the addition of 0·0015 cc. of rennet.

The horse serum in question was a normal horse serum, the durability of which was increased by the addition of some thymol crystals.

All experiments were made in test-tubes, the inner wall of which was covered with a thin film of paraffin in order to prevent alkali from the glass affecting the rennet-serum complex. All liquids used for the experiments were also kept in paraffined tubes, or tubes of Jena-glass.

Table I shows the inhibiting power of normal horse serum at 20°.

TABLE I.

	Time during which the rennet-serum complex is kept at 20° before milk is added to it								
	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
The maximum amount of rennet neutralised by 0·1 cc. of normal horse serum	0·04 cc.	0·04 cc.	0·06 cc.	0·07 cc.	0·08 cc.	0·09 cc.	0·09 cc.	0·09 cc.	0·09 cc.

The comparatively large amount of rennet neutralised at the moment of admixture, and which is not increased within the following 15 minutes, represents an actual change within this short period, and is not due to a continuation of the process when the samples were stored at 0° over night. The quantity of rennet neutralised at 0° is actually very small. An experiment, in which liquids, glasses, pipettes, etc., were kept at 0°, showed that the greatest quantity of rennet, neutralised by 0·1 cc. of normal horse serum under these circumstances, amounted to 0·005 cc.

Table I shows that the time during which the serum acts on rennet is not without importance for the inhibiting power of normal horse serum at 20°. This agrees fully with Hedin's observations. According to Korschun's views, however, the objection may be raised, that the effect of the pseudo-anti-rennet sets in at 20°. To examine the inhibiting power of the true antibody in the horse serum, it is therefore necessary to use a method other than that employed in Experiment 1.

Korschun has shown that the two inhibiting substances in the normal horse serum can be separated by dialysing the serum. As distinct from the true antibody, the pseudo-antirennet is dialysable. A dialysed horse serum should therefore give a measure only of the genuine antibody's mode of action.

As seen in Table I normal horse serum instantaneously neutralises a considerable quantity of rennet—0·04 cc. This quantity undoubtedly is

neutralised by the true antibody. A dialysed serum should therefore be capable of destroying a corresponding quantity of rennet in an equally short time, and thereby have reached the maximum of its inhibiting power. As can be seen from Tables II and III, this is by no means the case.

In these tables the results are given of two experiments, made with two samples of horse serum, the one dialysed longer than the other. For the experiment described in Table II, 10 cc. of the horse serum were dialysed for 48 hours in an animal membrane ("Fischblase") against running tap-water. To avoid bacterial action some thymol crystals were added to the serum. During the dialysis the volume of the serum increased to 14 cc. The precipitated euglobulin was separated from the dialysed serum by centrifuging. This euglobulin was also tested for inhibiting power, but proved to be without effect. Korschun has made the same observation. Half of the dialysed serum was used for the experiment, mentioned in Table II, while the remaining part—7 cc.—was brought back into the membrane and dialysed for further 48 hours. The volume of this second part thereby increased to 10·5 cc. This serum was used for the experiment in Table III.

In Fig. 1 the inhibiting powers of the dialysed sera are graphically shown, and compared with the inhibiting power of normal horse serum at 37° (Table IV). Like all the curves in this paper, those in Fig. 1 indicate the effect of the inhibiting substance in question, expressed by the ratio between the maximum amount of rennet, neutralised by the inhibiting substance, and the minimal quantity of rennet (i.e. 0·0015 cc.), required for the coagulation of 10 cc. of the milk used, without any addition of serum.

TABLE II.

	Time during which the rennet-serum complex is kept at 37° before milk is added to it								
The maximum amount of rennet neutralised by 0·14 cc. of a horse serum, dialysed for 48 hours	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
	0·02 cc.	0·08 cc.	0·12 cc.	0·14 cc.	0·16 cc.	0·16 cc.	0·16 cc.	0·16 cc.	0·16 cc.

As seen above, 0·14 cc. horse serum dialysed for 48 hours is equal to 0·1 cc. of normal horse serum.

TABLE III.

	Time during which the rennet-serum complex is kept at 37° before milk is added to it								
The maximum amount of rennet neutralised by 0·21 cc. horse serum, dialysed for 96 hours ..	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
	0·015 cc.	·06 cc.	0·11 cc.	0·13 cc.	0·15 cc.	0·15 cc.	0·15 cc.	0·15 cc.	0·15 cc.

0·21 cc. of the horse serum dialysed for 96 hours corresponds to 0·1 cc. normal horse serum.

The curves in Fig. 1 show perhaps more clearly than the corresponding tables that the period for which rennet and dialysed serum are kept before the milk is added to the complex is of decisive importance for the inhibiting power of these sera. The dialysis therefore does not differentiate between the true antibody and the pseudo-antirennet with the loss of the latter, but decreases

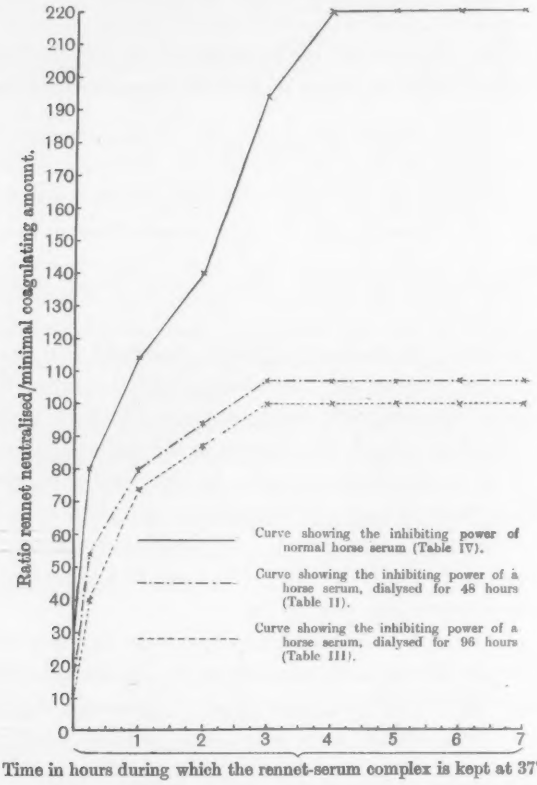

Fig. 1. Showing the inhibiting power at 37° of normal and dialysed horse serum.

the total inhibiting power of the serum, a decrease which is evidently greater the longer the dialysis lasts.

Even if the above data do not give any direct information as to the nature of the true antibody of horse serum, two conclusions may be drawn from them. Firstly, that this body cannot be a true antibody in the strict sense of the term, since the power of a true antibody is not decreased by dialysis

of the serum in which it is contained[1]. And secondly, they show that there is no such contrast between "true antibody" and "pseudo-antirennet" in their relations to dialysis, as Korschun supposed, which to him was one of the essential arguments for regarding them as two different substances.

(*b*) *Pseudo-antirennet; its mode of action and its nature.*

When a normal horse serum acts on rennet at 37° its inhibiting effect is much greater than at 20°, as shown in the following table (see Fig. 1).

TABLE IV.

	Time during which the rennet-serum complex is kept at 37° before milk is added to it								
	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
The maximum amount of rennet neutralised by 0·1 cc. of normal horse serum	0·04 cc.	0·12 cc.	0·17 cc.	0·21 cc.	0·29 cc.	0·33 cc.	0·33 cc.	0·33 cc.	0·33 cc.

This observation also agrees with Hedin's statement that the inhibiting power of the serum increases up to a certain equilibrium proportionally, not only to the time, but also to the temperature at which the serum acts on the rennet. Korschun regards this increase in the inhibiting power as being due to the so-called pseudo-antirennet. As this body is supposed to be extremely thermostable, it ought to be separated from the thermolabile "true antibody" by heating the horse serum. An experiment made with a heated serum therefore should show the effect of the pseudo-antirennet better than the normal horse serum was able to do.

Following Korschun's instructions, an experiment like this is best made with a horse serum diluted in the proportions (1 + 5) with distilled water or tap-water, and then boiled for some time. In making the experiment, 10 cc. of the above-mentioned horse serum were therefore mixed with 50 cc. of distilled water, and the mixture kept in a boiling water-bath for one hour. The inhibiting effect of the serum dilution thus treated was determined in the usual way and found to have the following values.

[1] The only apparent cause for a decrease in the power of a true antibody would be that the dialysis had deprived the serum, in which the antibody is contained, of its content of NaCl. But even if NaCl be added to the dialysed horse serum the inhibiting power of the latter is not altered.

TABLE V.

	Time during which the rennet-serum complex is kept at 37° before milk is added to it								
	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
The maximum amount of rennet neutralised by 0·6 cc. of a boiled horse serum dilution (1 + 5)	0·004 cc.	0·08 cc.	0·20 cc.	0·29 cc.	0·31 cc.	0·31 cc.	0·31 cc.	0·31 cc.	0·31 cc.

0·6 cc. of the boiled serum dilution contains 0·1 cc. horse serum.

The use of so large a quantity of serum dilution made the precipitation of the casein, formed in the samples by the action of the rennet, extremely difficult, as the dilution had lowered the concentration of the calcium salts in the milk too much. Normal conditions were, however, easily brought about by adding some drops of a calcium chloride solution (4 %).

This experiment plainly shows that by heating the horse serum a considerable decrease is effected in the amount of rennet instantaneously neutralised by the true antibody. On the other hand, the heating does not appear to have changed the inhibiting power of the pseudo-antirennet, as the quantity of rennet neutralised at 37° by the heated serum practically agrees with what is destroyed at the same temperature by normal horse serum (Table IV). Notwithstanding this, it would be erroneous to conclude, as Korschun does, that the heating has destroyed the true antibody without hurting the pseudo-antirennet. A closer examination of the conditions, obtaining in the experiment to which Table V refers, shows that a horse serum, diluted (1 + 5) and heated to 70° for a quarter of an hour, has a higher inhibiting power at 37° than the boiled dilution and that an unheated dilution (1 + 5) has a still greater effect at the same temperature. By heating a serum dilution therefore we weaken its total inhibiting power, also that part of it which is due to the pseudo-antirennet. Tables VI and VII and Fig. 2 illustrate this.

TABLE VI.

	Time during which the rennet-serum complex is kept at 37° before milk is added to it								
	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
The maximum amount of rennet neutralised by 0·6 cc. of a horse serum dilution (1+5), heated to 70° for a quarter of an hour ..	0·006 cc.	0·09 cc.	0·21 cc.	0·29 cc.	0·32 cc.	0·35 cc.	0·38 cc.	0·38 cc.	0·38 cc.

0·6 cc. of the dilution used contained 0·1 cc. horse serum.

TABLE VII.

	Time during which the rennet-serum complex is kept at 37° before milk is added to it								
	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
The maximum amount of rennet neutralised by 0·6 cc. of a horse serum dilution (1+5)..	0·04 cc.	0·12 cc.	0·27 cc.	0·33 cc.	0·37 cc.	0·40 cc.	0·42 cc.	0·42 cc.	0·42 cc.

0·6 cc. of the dilution used contained 0·1 cc. horse serum.

The heating has thus considerably damaged both the true antibody and the pseudo-antirennet, with the difference that the destruction of the first

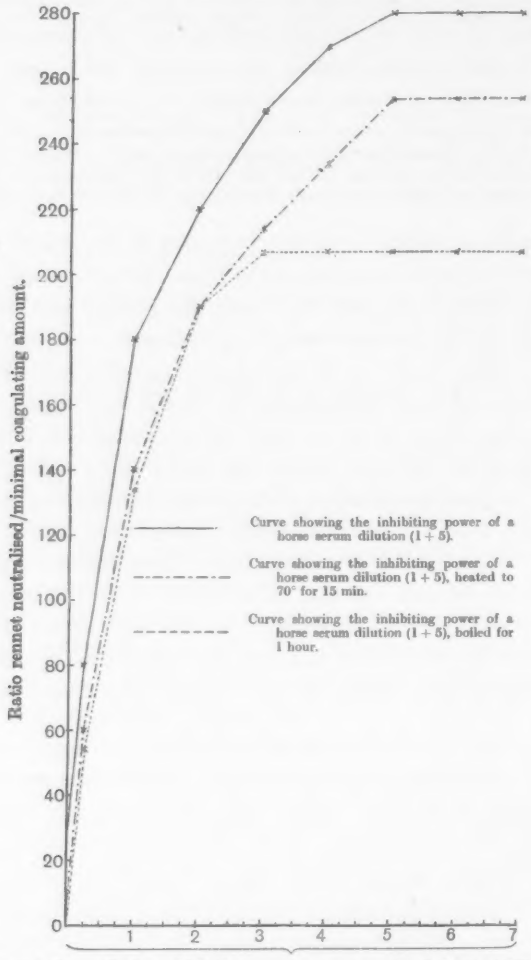

Fig. 2. Showing the inhibiting power at 37° of heated and unheated horse serum dilutions (1 + 5).

is, so to speak, complete, while the pseudo-antirennet is still active to a high degree and cannot be entirely destroyed. Then it must be assumed that the destruction of the pseudo-antirennet has practically come to an end, since a

serum dilution, boiled for one hour, gives values for its inhibiting power which are but little less than those shown by a dilution heated for a quarter of an hour to 70°. This partial destruction of the pseudo-antirennet and the increase in the inhibiting power by diluting it would hardly be explainable if the pseudo-antirennet, as supposed by Korschun, were a substance present in normal horse serum as an independent body. On consideration the above-mentioned facts rather indicate that the term pseudo-antirennet, as applied to the inhibiting power of heated and unheated serum dilutions, covers at least three properties of normal horse serum, i.e. its faculty of regulating the $[H^{\cdot}]$ in its dilutions, its alkalinity and its thermolability.

The importance of the first of these properties is apparent on an examination of the effect which dilution of normal horse serum has on its inhibiting power. Allusion has already been made to Hedin's statement that dilution of the normal serum does not change its inhibiting power; this is not in conformity with my own experiments. In view of the method employed by Hedin in his experiments, it is, however, impossible to attach importance to his deductions. By using Korschun's exact methods it is obvious that the inhibiting power is increased considerably by dilution of the serum. This may be seen from Tables VIII and IX, which concern two experiments made with diluted horse sera.

TABLE VIII.

	Time during which the rennet-serum complex is kept at 37° before milk is added to it								
	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
The maximum amount of rennet neutralised by 0·3 cc. of a horse serum diluted (1+2)..	0·04 cc.	0·12 cc.	0·25 cc.	0·28 cc.	0·31 cc.	0·36 cc.	0·36 cc.	0·36 cc.	0·36 cc.

0·3 cc. of the serum dilution used contained 0·1 cc. horse serum.

TABLE IX.

	Time during which the rennet-serum complex is kept at 37° before milk is added to it								
	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
The maximum amount of rennet neutralised by 0·6 cc. of a horse serum diluted (1+5)..	0·04 cc.	0·12 cc.	0·27 cc.	0·33 cc.	0·37 cc.	0·40 cc.	0·42 cc.	0·42 cc.	0·42 cc.

0·6 cc. of the serum dilution used contained 0·1 cc. horse serum.

This increase in the inhibiting power becomes more apparent on examination of the $[H^{\cdot}]$ of the normal horse serum and its dilutions. Tested electrically the $[H^{\cdot}]$ of the normal horse serum in question gives the value

$p_{H\cdot} = 7{\cdot}99$. Diluting the serum (1 + 5) its alkalinity does not decrease. On the contrary, measured at 37° its [H˙] gives values which vary from $p_{H\cdot} = 8{\cdot}20$ to $p_{H\cdot} = 7{\cdot}90$, depending on the time at which they are measured, either directly the dilution is made or later. The [H˙] of the dilution (1 + 2) agrees, as does that of the dilution (1 + 5), with the [H˙] of the normal horse serum.

Considering the extreme sensitiveness of rennet towards even very small quantities of alkali, it seems natural to connect the increase of the inhibiting power of the dilutions with the tendency of the serum to maintain normal [H˙] even in its higher dilutions. Indeed we know that the amount of rennet inhibited by an alkali solution is dependent on the [H˙] of this solution only, and not on the exact number of H- or OH-ions present. The same fact must therefore determine the action of the serum dilutions if their [H˙] has any influence on their increased inhibiting power. From this point of view it is, however, also justifiable to suppose that a greater quantity, either of the alkaline solution or of the serum dilutions, will destroy more rennet than a smaller amount is able to do. To explain this we may suppose that 0·1 cc. of alkaline solution of a certain [H˙] neutralises 0·02 cc. rennet solution; 0·6 cc. of the same alkaline solution should then neutralise $6 \times 0{\cdot}02$ cc. = 0·12 cc. rennet. That this occurs when the alkali is kept in six tubes each containing 0·1 cc. is obvious. If 0·6 cc. of the alkali is brought into one single tube, the same amount of rennet should be inhibited, as in this case also we have 0·02 cc. rennet corresponding to each of the alkali's six units of 0·1 cc. I have tried experimentally to ascertain how far this agrees with the actual, and found that 0·6 cc. of a Na_2HPO_4 solution of a certain [H˙] inhibits seven times more rennet than 0·1 cc. of the same phosphate solution. This result is very satisfactory, as experimental errors must always be considerable in work of this kind.

If a greater quantity of alkali is thus able to destroy more rennet than a smaller, the serum and its dilutions must do the same, as their [H˙] is also constant; and 0·6 cc. of a serum dilution (1 + 5) must therefore inhibit more rennet than 0·3 cc. serum dilution (1 + 2), and this 0·3 cc. again more than 0·1 cc. of normal horse serum. The question whether the increase in the inhibiting power of the serum can be due to its property of maintaining normal [H˙] in its dilutions therefore resolves itself into one, as to whether an increase in the inhibiting power, equal to that shown by the serum dilutions, can be caused by an alkaline solution of the same [H˙] as the serum—$p_{H\cdot} = 7{\cdot}99$ (at 37°)—and used in the same quantities as this.

The increase in the inhibiting power of the serum dilution (1 + 2) is equal

to 0·03, this value being the difference between the maximum amount of rennet (0·33 cc.) inhibited by 0·1 cc. of normal horse serum at 37° and the greatest quantity (0·36 cc.) destroyed at the same temperature by 0·3 cc. of the serum dilution (1 + 2).

The increase in the inhibiting power of the solution (1 + 5) is 0·06, as 0·3 cc. of the dilution (1 + 2) inhibits 0·36 cc. rennet, and 0·6 cc. of the dilution (1 + 5) inhibits 0·42 cc.

As regards the alkaline solution, it has already been pointed out that the increase in its inhibiting power is proportional to the amount of solution used. 0·3 cc. alkaline solution of a certain [H˙] therefore inhibits three times as much rennet as 0·1 cc. of the same solution. 0·1 cc. alkali of the concentration required[1] inhibits 0·04 cc. rennet, and 0·3 cc. of this alkali therefore ought to inhibit $3 \times 0{\cdot}04 = 0{\cdot}12$ cc. rennet, giving an increase in the inhibiting power equal to $0{\cdot}12 - 0{\cdot}04 = 0{\cdot}08$. On the other hand, 0·6 cc. of the alkali in question[2] will inhibit $6 \times 0{\cdot}04 = 0{\cdot}24$ cc. rennet, which represents an increase in the inhibiting power equal to $0{\cdot}24 - 0{\cdot}12 = 0{\cdot}12$. The experiments upon which these calculations are based are somewhat crude and the values cannot be regarded as absolutely exact. Nevertheless they suffice to show that an increase in the inhibiting power such as is shown by the serum dilutions is to be expected when they are used in increasing quantities.

With respect to the other properties which are necessary for the action of the pseudo-antirennet, the alkalinity and the thermolability of the serum, their importance is best shown by a closer examination of the inhibiting power of a boiled horse serum.

In view of the results of the above-mentioned experiments one might expect the reduction of the inhibiting power of the heated serum dilutions to be due to a decrease of their [H˙] caused by the treatment. This, however, was not the case, as the [H˙] of the boiled serum dilution gave roughly the same value as that shown by the unheated dilutions. Nevertheless there is an important relation between the inhibiting power of the boiled serum and its [H˙]. This became evident by a determination of the inhibiting power of 0·6 cc. of the phosphate solution at 37°. The experimental data are given in Table X.

[1] A solution of Na_2HPO_4 and KH_2PO_4 was used, made according to Sörensen's [1909] instructions by mixing 9·75 cc. $n/15$ Na_2HPO_4 and 0·25 cc. $n/15$ KH_2PO_4; tested at 18° this mixture has the $[H^{\cdot}]p_H = 8{\cdot}038$.

[2] In an experiment already mentioned 0·6 cc. phosphate solution neutralised 0·28 cc. rennet, and not 0·24 cc.

TABLE X.

	Time during which the rennet-phosphate complex is kept at 37° before milk is added to it								
	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
The maximum amount of rennet neutralised by 0·6 cc. of a phosphate solution with the $[H^{\cdot}]p_{H^{\cdot}} = 8{\cdot}038$ (18°) . .	0·003 cc.	0·04 cc.	0·19 cc.	0·23 cc.	0·28 cc.	0·28 cc.	0·28 cc.	0·28 cc.	0·28 cc.

As can be seen from the curves in Fig. 3 there is a striking agreement between the inhibiting effects of the phosphate solution and the boiled serum

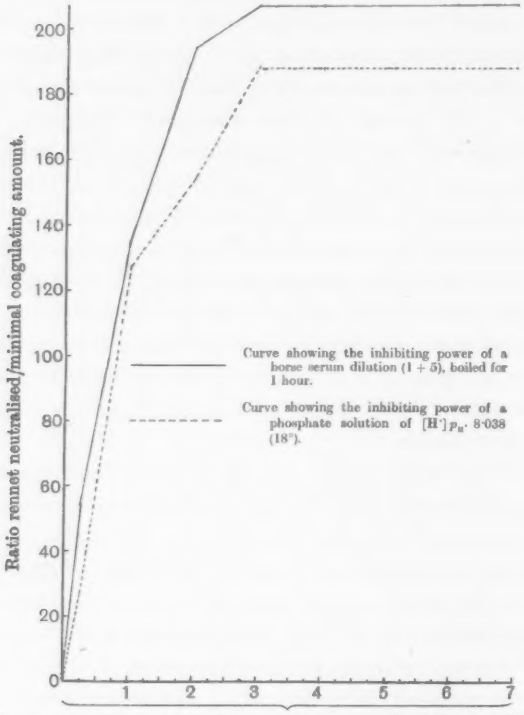

Fig. 3. Showing the inhibiting power at 37° of a boiled horse serum dilution (1+5) and of a phosphate solution of nearly the same $[H^{\cdot}]$.

dilution. There appears no reason to doubt that this agreement is due to the fact that the inhibiting power of the boiled horse serum is an effect of its $[H^{\cdot}]$ only. This will be sufficient to indicate the importance of the alkalinity

of the serum with respect to its inhibiting power. It therefore only remains to be shown that the inhibiting power is dependent also on the thermolability of the serum.

The value of the [H˙] is clearly as important for the inhibiting power of the serum dilutions as it was found to be for the action of the boiled serum. The inhibiting power of the dilution (1 + 5), for instance, must therefore be regarded as a mixed effect of its [H˙] and of various other supplementary properties (substances?). In addition to the effect of the [H˙] the inhibiting power of this dilution therefore also shows the effect of these properties. As can be seen from Table XI they also relate to the pseudo-antirennet, as their inhibiting power increases proportionally to the time the serum acts on the rennet.

TABLE XI.

	Time during which the serum or the phosphate solution acts on the rennet at 37°								
	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
The maximum amount of rennet neutralised by 0·6 cc. of a serum dilution (1+5)	0·04 cc.	0·12 cc.	0·27 cc.	0·33 cc.	0·40 cc.	0·42 cc.	0·42 cc.	0·42 cc.	0·42 cc.
The maximum amount of rennet neutralised by 0·6 cc. of a phosphate solution of the same [H˙] as the horse serum	0·003 cc.	0·04 cc.	0·19 cc.	0·23 cc.	0·28 cc.	0·28 cc.	0·28 cc.	0·28 cc.	0·28 cc.
The inhibiting effect of the supplementary properties of the horse serum	0·037 cc.	0·08 cc.	0·08 cc.	0·10 cc.	0·12 cc.	0·14 cc.	0·14 cc.	0·14 cc.	0·14 cc.

Calculations similar to that made in Table XI show that the effect of these supplementary properties of the serum dilution (1 + 5) is greatly reduced by heating it to 70°. In a boiled dilution their effect has completely disappeared. As the thermolability of the serum is thus decisive for the effect of these properties, it must also be of importance for the action of the pseudo-antirennet, since the properties in question, as shown in Table XI, are part of the pseudo-antirennet.

Some interesting light as to the nature of these supplementary properties, or substances, of normal horse serum may be obtained from the experiments already discussed. As they cannot be demonstrated in a boiled horse serum dilution, their effect is obviously due to the proteins of the serum, or perhaps to their colloid qualities. Moreover, the effect of the properties in question increases proportionally to time, and to the temperature at which the serum acts on rennet. This can be seen from Tables I and XII. Table I showed the inhibiting power of 0·1 cc. of normal horse serum at 20°. But the figures

at the same time also practically show the inhibiting effect of the supplementary properties at 20°, as the [H·] of 0·1 cc. of normal horse serum is hardly of any importance for the amount of rennet destroyed at this temperature. Experiments showed that 0·1 cc. of the phosphate solution neutralised 0·003 cc. rennet at 20°, an amount which does not increase even if the solution acts on rennet for several hours.

The inhibiting power of the supplementary properties at 37° is shown in Table XII.

TABLE XII.

	Time during which the serum or the phosphate solution acts on the rennet at 37°								
	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
The maximum amount of rennet neutralised by 0·1 cc. of normal horse serum	0·04 cc.	0·12 cc.	0·17 cc.	0·21 cc.	0·29 cc.	0·33 cc.	0·33 cc.	0·33 cc.	0·33 cc.
The maximum amount of rennet neutralised by 0·1 cc. phosphate solution of the same [H·] as the horse serum	0·003 cc.	0·02 cc.	0·04 cc.	0·04 cc.	0·04 cc.	0·04 cc.	0·04 cc.	0·04 cc.	0·04 cc.
The effect of the supplementary properties of the horse serum is therefore equal to	0·037 cc.	0·10 cc.	0·13 cc.	0·17 cc.	0·25 cc.	0·29 cc.	0·29 cc.	0·29 cc.	0·29 cc.

The effect of these properties is thus considerably increased by raising the temperature at which the experiment is made.

Furthermore, some light is thrown on them from Table XII. Compared with the figures in Table XI, which showed their inhibiting power at high dilution, the figures in Table XII indicate that their effect is greater when they act on rennet in concentrated than in diluted form.

The real cause of the appearance of these properties, or substances, cannot be determined from the experiments discussed above. Korschun's "true antibody" is certainly either identical with them or part of them. Nevertheless the use of this expression for the explanation of the action of the normal serum on rennet is not to be recommended. This action cannot be due to a true antibody, but is more probably attributable to physico-chemical effects. On the other hand, Hedin's conclusion that this action is a mere adsorption must undoubtedly only be a partial explanation.

II. The Inhibiting Effect of Normal Rabbit Serum on Rennet.

As can be seen from the following table the action of normal rabbit serum on rennet has the same characteristics as that of normal horse serum, with the difference merely that its effect is distinctly weaker.

TABLE XIII.

No.	Material	Temp.	Vol. of serum	Maximum amount (in cc.) of rennet neutralised when the rennet-serum complex is kept for the following times before milk is added to it								
				0	0·25	1	2	3	4	5	6	7 hours
1	Normal rabbit serum	20°	0·1 cc.	0·004	0·008	0·01	0·015	0·03	0·03	0·03	0·03	0·03
2	Normal rabbit serum	37°	0·1 cc.	0·004	0·025	0·085	0·11	0·14	0·17	0·17	0·17	0·17
3	Normal rabbit serum dialysed for 48 hours through an animal membrane	37°	0·35 cc. of the dialysed serum (=0·1 cc. undialysed serum)	0·0015	0·02	0·07	0·09	0·15	0·17	0·17	0·17	0·17
4	Rabbit serum diluted (1+5)	37°	0·6 cc. diluted serum (=0·1 cc. normal serum)	0·004	0·025	0·09	0·13	0·16	0·18	0·21	0·21	0·21
5	Rabbit serum diluted (1+5) and heated to 70° for 0·25 hour	37°	0·6 cc. diluted serum (=0·1 cc. undiluted serum)	0·0015	0·006	0·12	0·15	0·15	0·15	0·15	0·15	0·15

The experiments from which these numbers are derived were arrived at in the same way as those dealing with the inhibiting power of normal horse serum. The same rabbit serum was used for all experiments. In addition two other rabbit sera were tested, one of which showed a lower inhibiting power than that used for the experiments quoted. The intensity of the inhibiting power thus varies not only with different species of animals, but also with individuals of the same species, a fact which has already been recognised.

III. The Inhibiting Power of a Rennet Antiserum.

The question of the possible identity of artificial antirennet and that found in normal horse serum has already been referred to in the introduction. Previous researches, Korschun's and Morgenroth's [1900] especially, appeared to point in this direction, at any rate as far as those rennet antisera were concerned, which were made by immunising animals, e.g. goats or rabbits, with a true rennet enzyme. The antirennet of the normal serum, or at least a part of it, was regarded as a true antibody, formed in the serum of the animal in question by autoimmunisation with rennet, originating from the mucous membranes of its stomach. As the results of the experiments mentioned in

this paper affect this conception of the inhibiting effect of normal horse serum in various respects, it appeared of interest to ascertain whether the action of the rennet antiserum could be explained in the same manner as that of the normal serum, or whether in this antiserum there occurred a typical antibody with properties similar to those claimed by Korschun for the antibody of normal horse serum.

The rennet antiserum, employed in the experiments discussed below, was obtained in the following manner.

The immunisation was accomplished in The Swiss Institute for Dairy Bacteriology, Liebefeld near Berne. As antigen a pure rennet powder (Merck) with the coagulating power $1-1{\cdot}5 \times 10^6$ was used. The animals were injected with an extract of this rennet powder in the strength of 1·0 g. of rennet powder to 10 cc. of sterile distilled water. For each injection, given every other day, a fresh quantity of extract was made. At the beginning of the treatment the rabbits used for the immunisation did not show any loss of weight, but later on, after the seventh injection, a rather heavy fall could be noticed. Two of the rabbits died at the end of the treatment with typical anaphylactic symptoms. The subcutaneous injection caused a somewhat considerable local infiltration. Contrary to what was expected, the rennet antiserum was found to be very durable. Even at the end of a year its inhibiting power was entirely unimpaired.

Unfortunately it has been necessary to limit the number of experiments originally planned with this antiserum, as the quantity available was altogether inadequate, especially as each experiment had to be carried out two or three times.

It is interesting to note that the inhibiting power of the rennet antiserum at 20° and 37° agrees well with the properties claimed by Korschun for a serum containing a true antirennet. See Table XIV.

TABLE XIV.

The inhibiting power of rennet antiserum at 20° *and* 37°.

	Time during which the rennet-serum complex is kept before milk is added to it								
1.	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
The maximum amount of rennet neutralised by 0·1 cc. of a rennet antiserum, at 20°	0·03 cc.	0·03 cc.	0·03 cc.	0·03 cc.	0·03 cc.	0·03 cc.	0·03 cc.	0·03 cc.	0·03 cc.
2. The maximum amount of rennet neutralised by 0·1 cc. of a rennet antiserum, at 37°	0·03 cc.	0·045 cc.	0·065 cc.	0·09 cc.	0·115 cc.	0·13 cc.	0·13 cc.	0·13 cc.	0·13 cc.

At 20° the antiserum thus neutralises a considerable quantity of rennet, which does not increase even if the rennet-antiserum complex is kept for seven hours at 20°. Furthermore, the effect of the true antirennet, i.e. the capacity of the antiserum for destroying a maximum amount of rennet in a very short time (a few seconds), has considerably increased owing to the immunisation, whereas the effect of the pseudo-antirennet at 37° is still unaltered, as the difference in the inhibiting power between the pseudo-antirennet of the normal rabbit serum and that of the antiserum (Tables XIII, No. 2, and XIV, No. 2) is no doubt due to individual differences in the inhibiting power of sera of the same species, and not to a decrease in the effect of the pseudo-antirennet owing to the dialysis.

At this point, however, the agreement between the effect of the antiserum and the properties claimed by Korschun for the true antirennet ceases. Testing the effect of the antiserum under other conditions, the agreement rather seems to have been accidental. Dialysis, e.g., causes a decrease in the entire inhibiting power of the antiserum (Table XV), just as was the case with the dialysed horse serum.

TABLE XV.

	Time during which the rennet-serum complex is kept at 37° before milk is added to it								
The maximum amount of rennet neutralised by 0·22 cc. of a rennet antiserum dialysed for 48 hours	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
	0·01 cc.	0·03 cc.	0·05 cc.	0·08 cc.	0·08 cc.	0·08 cc.	0·08 cc.	0·08 cc.	0·08 cc.

3 cc. of antiserum were dialysed for 48 hours, its volume thereby increasing to 6·5 cc. 0·22 cc. of the dialysed antiserum therefore is equal to 0·1 cc. untreated antiserum.

This decrease in the inhibiting power of the antiserum is not consistent with the presence of a true antibody in this serum.

Similarly, it is noteworthy that the effect of the pseudo-antirennet is increased far more by diluting the antiserum (Table XVI) than by diluting normal rabbit serum (Table XIII, No. 4). This would hardly be the case if the pseudo-antirennet, as assumed above, had not been altered by the immunisation.

TABLE XVI.

	Time during which the rennet-serum complex is kept at 37° before milk is added to it								
The maximum amount of rennet neutralised by 0·6 cc. of a rennet antiserum diluted (1 + 5)	0 min.	15 min.	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours
	0·01 cc.	0·06 cc.	0·20 cc.	0·22 cc.	0·23 cc.	0·24 cc.	0·24 cc.	0·24 cc.	0·24 cc.

0·6 cc. of the diluted antiserum contained 0·1 cc. antiserum.

The increase in the effect of the pseudo-antirennet is more explicable if viewed from the same standpoint as the changes in the inhibiting power of normal horse serum caused by dilution. Unfortunately, the experiments made with the antiserum had not left a sufficient quantity for a determination of its [H˙]. Using Sörensen's indicator method it was, however, found that the antiserum was more alkaline than normal rabbit serum, but less alkaline than normal horse serum[1]. Dilutions of the antiserum therefore have a smaller [H˙], i.e. a higher alkalinity, than dilutions of normal rabbit serum, as the sera are able to maintain normal [H˙] even in high dilutions. As a matter of course the dilutions of the antiserum therefore must destroy more rennet than dilutions of normal rabbit serum.

There is another peculiarity in the inhibiting effect of the antiserum at 20°, which is not so easily explained as the changes in the action of the pseudo-antirennet. As can be seen from Tables XIII, No. 1, and XIV, No. 1, the maximum amount of rennet neutralised by normal rabbit serum at 20° is equal to that destroyed by the antiserum at the same temperature. The velocity of reaction of the destruction is, however, very different for the two sera, the antiserum neutralising the maximum amount in a very short time, normal rabbit serum, on the contrary, in four hours only. As regards the diluted antiserum, the velocity of reaction is less than that of the antiserum itself (Table XVI).

Changes in the velocity of reaction, such as are shown by the antiserum, are well known from the study of the action of adsorbing bodies [Bayliss, 1906]. As was the case for the antibody, these changes are proportional to the concentration of the adsorbing body; adsorption increasing with the concentration. The peculiarity of the inhibiting effect referred to above is therefore possibly due to an increase in the concentration of some substance in the serum during the immunisation.

The researches on the inhibiting power of the antiserum here discussed do not favour the belief that the action of the rennet antiserum is of a special nature. On the contrary, they appear to indicate that this action cannot be due to the presence of a true antibody formed in the serum during immunisation.

[1] This is remarkable, as in his researches on the [H˙] of the normal serum Michaelis [1914] points out that changes in the normal [H˙] of the serum are extremely infrequent, at least as far as an increase is concerned.

Conclusions.

1. The inhibiting effect of normal horse serum on the coagulating power of the rennet enzyme is not, as supposed by Korschun, due to two inhibiting substances, the so-called true antibody and the pseudo-antirennet. Neither can Hedin's explanation of this effect as a mere adsorption be regarded as fully satisfactory.

2. It can be shown furthermore that the [H˙] of normal horse serum is highly important for its inhibiting power.

3. Normal rabbit serum shows the same characteristics in its effect on rennet as normal horse serum.

4. The inhibiting power of a rennet antiserum made by immunising rabbits with rennet enzyme can hardly be due to the presence of a true antibody, as this serum also tends to show the characteristics of normal horse serum.

The author takes the opportunity of expressing his thanks to Prof. R. Burri, head of The Swiss Institute for Dairy Bacteriology, Liebefeld near Berne, who placed the standardised rennet solution at his disposal. He is also very indebted to Dr E. Atkin, of the Lister Institute, for the determinations of [H˙] mentioned in this paper.

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XIII. COUNTER DIFFUSION IN AQUEOUS SOLUTION.

By GEORGE STANLEY WALPOLE.

From the Wellcome Physiological Research Laboratories, Herne Hill, London, S.E.

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In the introduction to a description of some experiments published in this Journal W. A. Osborne and Miss L. C. Jackson [1914] have written: "If, for instance, there are two solutions one vertically placed above the other and in contact over a relatively small surface, and if both contain decinormal sodium chloride and one of them, necessarily the lower, contains ammonium sulphate of three molecular strength, then after some days' diffusion it is found that the sodium chloride concentration in the upper fluid is greater than decinormality whilst that in the lower fluid is correspondingly reduced."

In describing their analytical results the authors show that these conclusions were based solely on Cl and SO_4 determinations and that it was assumed that Cl represented NaCl and SO_4 represented $(NH_4)_2SO_4$.

Realising that incorrectness in this assumption might permit the establishment of these observations as interesting extensions of already recognised diffusion phenomena, I repeated this experiment, supplementing the Cl and SO_4 determinations by those of K and (NH_4). As a matter of convenience KCl was used instead of NaCl. The results, given below, confirm in every respect Osborne and Jackson's analytical determinations, but the additional data with regard to K and (NH_4) lead me to suggest the following alterations in the conclusions drawn: "...then after some days' diffusion it is found that the ammonia has diffused into the upper fluid faster than the equivalent quantity of sulphate, with the result that chloride has increased in concentration in the upper fluid while sodium (potassium) has increased in concentration in the lower fluid."

The experiments described in this note were chosen with the view of establishing the phenomenon as one resulting from the varying velocities of ionic diffusion.

Experimental.

To simplify explanation it is convenient to refer to the originally evenly distributed solute as the *test substance*, and the solute added to one solution only as the *diffusing substance*; also, in the case of simple inorganic electrolytes to speak of them as if they were completely dissociated.

The method of experiment devised by Osborne and Jackson was followed very closely. The volume of fluid above the tap was, as in their case, equal to that of the bore of the tap plus that below the tap. This was 15·75 cc. in some experiments and 17·5 cc. in others. Osborne used larger volumes in his experiments, an arrangement which demands longer diffusion periods for the same ionic differentiation of the test substance, but permits, because of the larger volumes of material provided, more accurate analytical determinations in the fluids after diffusion.

The bottom fluid is analysed before diffusion as a check on the analytical methods. After diffusion each was well mixed and analysed in the same manner.

Experiment 1.

Test substance 0·15 *N KCl*; *diffusing substance* 0·790 *N* HNO_3.

Volume of top fluid = volume of bottom fluid = 15·75 cc.

Before diffusion		K	Cl	H	NO_3
	Top fluid	0·1508 N	0·1508 N	—	—
	Bottom fluid	0·1508 N	0·1508 N	0·790 N	[0·790 N]
After diffusion for nine days					
	Top fluid	0·1388 N	0·1625 N	0·1250 N	[0·1013 N]
	Bottom fluid	0·1627 N	0·1398 N	0·6610 N	[0·6839 N]

The K was weighed as K_2SO_4; Cl weighed as AgCl; H by titration; NO_3 by difference, for the sum of the normalities of the anions and kations in any solution must be equal.

The figures above show that the H and NO_3 originally to be found only in the bottom fluid have diffused upwards into the top fluid but with unequal velocities. The H has moved faster than the NO_3. At the same time it is seen that the K and Cl originally both of them 0·1508 N throughout the whole system have diffused in such a fashion that K has increased in concentration in the lower fluid by 0·0119 N and Cl has increased in concentration in the upper fluid by almost exactly the same amount.

Experiment 2.

Test substance 0·15 *N KCl*; *diffusing substance* 0·25 *N* $Ba(OH)_2$.

Volume of top fluid = volume of bottom fluid = 15·75 cc.

Before diffusion		K	Cl	Ba	OH
	Top fluid	0·1508 N	0·1508 N	—	—
	Bottom fluid	0·1508 N	0·1508 N	0·2525 N	0·2525 N
After diffusion for 14 days					
	Top fluid	0·1603 N	0·1413 N	0·0238 N	0·0380 N
	Bottom fluid	0·1413 N	0·1591 N	0·2244 N	0·2060 N

Ba was weighed as $BaSO_4$ and K weighed as K_2SO_4 in filtrate. A correction was made for solubility of $BaSO_4$. Cl weighed as AgCl, OH by titration.

Here Ba and OH have diffused from the lower fluid which alone originally contained them into the upper fluid. The OH has moved the faster. With regard to the test substance KCl the K has increased in concentration by 0·0095 N in the top fluid and the Cl has increased by 0·0091 N, nearly the same amount, in the lower fluid.

Experiment 3.

Test substance 0·15 *N KCl*; *diffusing substance* 0·773 *N* NH_4OH.

Volume of top fluid = volume of bottom fluid = 17·5 cc.

Before diffusion		K	Cl	NH_4	OH
	Top fluid	0·1508 N	0·1508 N	0·7730 N	0·7730 N
	Bottom fluid	0·1508 N	0·1508 N	—	—
After diffusion for six days					
	Top fluid	0·1512 N	0·1500 N	0·7007 N	0·6971 N
	Bottom fluid	0·1521 N	0·1508 N	0·0723 N	0·0760 N

Here because of its lower specific gravity the solution containing the diffusing substance had to be placed on top. Although of three times the normality of the baryta used as diffusing substance the effect in upsetting the even distribution of K and Cl is so small as to be indistinguishable in the face of experimental error.

Experiment 4.

Test substance 0·15 *N KCl*; *diffusing substance* 3 *N* $(NH_4)_2SO_4$.

Volume of top fluid = volume of bottom fluid = 17·5 cc.

Before diffusion		K	Cl	NH_4	SO_4
	Top fluid	0·1508 N	0·1508 N	—	—
	Bottom fluid	0·1508 N	0·1508 N	2·91 N	2·91 N
After diffusion for 15 days					
	Top fluid	0·1391 N	0·1581 N	0·3365 N	0·2609 N
	Bottom fluid	0·1577 N	0·1434 N	2·6100 N	2·5933 N

NH_4 by Kjeldahl; SO_4 weighed as $BaSO_4$; Cl weighed as AgCl. The determination of such small quantities of K in the presence of so much ammonium sulphate could not be performed with accuracy; in driving off the $(NH_4)_2SO_4$ so that the K_2SO_4 remaining might be weighed a loss of two or three per cent. seemed unavoidable. This fact is reflected in the inconsistencies in the analytical figures for the fluids after diffusion.

The analytical figures of these four experiments, together with those of Osborne and Jackson, suggest the following generalisation with regard to diffusion experiments conducted in this fashion.

The ion of the test substance having the opposite sign to the faster ion of the diffusing substance accumulates in the direction of the diffusion; the other ion of the test substance accumulates in the other direction. When the diffusing substance is feebly ionised such change in the distribution in the ions of the test substance does not occur.

Further remarks in this note will, for this reason, refer only to Experiments 1, 2 and 4 above.

Theoretical.

In all solutions it is a necessary condition that at any part of the volume positive and negative ions must be present in amounts electrically compensating. Those solutions in which some process of natural diffusion is occurring or has occurred are, in view of the fact that they manifest no external electrical phenomena, no exception to this rule. When, therefore, an electrolyte diffuses into an unionisable solvent its two ions must diffuse in company whether they are dissociated or free. If they differ in intrinsic mobility the faster ion will tend to diffuse ahead of the other, but because of the intense electrical field so established will succeed in doing so only to an inappreciable degree. By breaking down this field, however, the process may be allowed to proceed. If this is done by the direct application of an electric field from external sources the process is *electrolysis*. If it is the result of a differential migration of the ions of another electrolyte introduced into the system whereby all potential gradients are neutralised it is *counter diffusion*.

At this point it will be well to distinguish between what may be deduced from first principles with regard to these experiments and what facts are learnt from the analytical results.

In experiments arranged in this manner, with the top and bottom fluids exactly equal in volume, the test substance equally distributed throughout, and the diffusing substance originally in the bottom fluid only, fundamental space relationships and the principle of electrical compensation demand:

(*a*) The sum of the normalities of the anions is equal to the sum of the normalities of the kations in each fluid both before and after the experiment. [This is true, of course, of every solution.]

(*b*) The change of normality of either ion of the test substance in the

top fluid, owing to diffusion, is equal and opposite in sign to the change of normality of the same ion in the bottom fluid.

(*c*) The sum of the changes of normality of the ions of the test substance in either fluid is equal but opposite in sign to the sum of the changes of normality of the ions of the diffusing substance in that fluid, if normalities of negatively charged ions are treated as negative quantities and the summation performed algebraically.

These three generalisations, deduced directly from first principles, provide an excellent check on analytical results. They may be represented in the general case by a set of simple simultaneous equations insufficient in number to determine absolutely the values of the unknown quantities.

The *experiments* made provide *further* information:

(α) That ion of the diffusing substance which is usually regarded as having the greater intrinsic velocity diffuses upwards to an extent greater than does the other; at least it does so in the three experiments above where the ions of the test substance, KCl, have approximately equal intrinsic velocities.

(β) The ratio of the nett migrations of the two ions of the diffusing substance is much less than the ratio of their accepted intrinsic velocities.

(γ) The migrations of K and Cl past the plane of the original boundary separating the two fluids are approximately equal; presumably because of their nearly equal migration velocities. These ions therefore share equally in the task of compensating for the unequal migrations of the ions of the diffusing substance.

It may be noted that it follows from the generalisations (*c*) and (β) above, that the nett migrations of the ions of the test substance past the plane of the original boundary must be smaller than the nett migrations of the ions of the diffusing substance.

Examples of all these generalisations may be seen by referring to the figures of Experiment 1 and those deduced therefrom given below:

	Test substance		Diffusing substance	
Change of normality of top fluid, i.e. nett migration past the plane of the original boundary	$K+$	$Cl-$	$H+$	NO_3-
	$-0{\cdot}0120$	$+0{\cdot}0114$	$+0{\cdot}1250$	$+0{\cdot}1013$
	(sum $= -0{\cdot}0234$)		(sum $= +0{\cdot}0237$)	

It follows, too, that when a binary electrolyte diffuses into water it becomes, to a minute extent, acid or alkaline according as the anion or kation of the diffusing substance is the faster. By adding litmus to

the water ahead of the diffusion front Durrant [1906] has observed the development of alkali in this manner by the diffusion of nitric acid.

Beyond those of Osborne and Jackson, who originated this method, I have not been able to find any descriptions of experiments where *differential* diffusion has been followed analytically. Durrant's observations [1906], although quantitative, were made rather on the distance migrated by different ions in the same time, and not upon the differences in quantity of the different ions passing a certain point.

The main interest in these results, however, lies in their relation to the theory of boundary potentials, for they represent a summation of the diffusions between the two liquids and, therefore, an integration of a function of the contact potential. The recognised complexity of these calculations will be reflected in any further attempt to predict from accepted data the results obtained above by analysis.

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XIV. THE DIGESTION AND ABSORPTION OF PROTEIN AND FAT IN NORMAL AND DEPANCREATISED ANIMALS.

By ERNEST WILLIAM HENDERSON CRUICKSHANK

(*Carnegie Research Fellow*).

From the Institute of Physiology, University College, London.

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Von Noorden [1893] and Rubner [1879] showed that on a meat or concentrated protein diet, the protein N was well absorbed, the utilisation figure varying from 90 to 98 per cent. These and several other workers have shown that within limits the greater the amount of protein given in a digestible form the better is its utilisation.

Within the limits of normal dietary there are several factors which influence the absorption of fat. Von Noorden's experiments [1907, 1] with butter fat given in varying amounts in an otherwise uniform diet show clearly that proportionately more fat is excreted when the food is almost fat free than when a great deal of fat is administered. With 4·2 g. fat in the food the utilisation was 42·9 per cent., while with 80·2 g. the figure rose to 93·6 per cent.

The physical consistency and the melting point of the fat are of importance as has been demonstrated by Munk [1884] and by Arnschink [1890], who have fully worked out the relation of the melting point to facility of absorption. Again, as Bucheim [1874] pointed out with regard to the ready utilisation of cod liver oil, the percentage of free fatty acid present in a fat exercises a marked influence. Blumfeld and Hauser [1888] obtained quite as good results with butter and milk fat as with preparations containing a certain percentage of free fatty acid.

As far back as 1851, Traube and others showed that it was possible in

diabetics to obtain a good utilisation of the food given. Hirschfeld [1891] in a paper on a new clinical form of diabetes stated that he could only recover 32 per cent. of the N ingested, while Brugsch [1906] found a loss of 20–25 per cent. of N and 50 to 60 per cent. of the fat ingested.

From a clinical point of view a considerable amount of work has centred around the question of the utilisation of fat in diabetes. E. Zunz and Mayer [1904] confirmed the statement that the closing of the pancreatic duct leads to a slowly ensuing disturbance of absorption. This impairment of function is slight compared with the disturbance following total extirpation with a diet containing no pancreas. In dogs, after total extirpation of the pancreas Abelmann [1890] found the following fat absorption figures: in small intestine non-emulsified fat, 32 per cent.; emulsified and milk fat, 80 per cent.; in the faeces on a milk diet there was a loss of 43–70 per cent. Müller [1887] and von Noorden [1907, 2] and others state that with disorders of pancreatic secretion it is the fat-splitting process which is affected. This view was opposed by Deucher, who obtained in severe disorders of the pancreas as complete a splitting of the fat as in the healthy organism, 62–80 per cent.

In the experiments described in this paper I have endeavoured to determine the amount of absorption which takes place in normal animals under a low and high protein and fat diet respectively, and to note what variation from the normal figures results when the animal is

(1) partially depancreatised (*a*) with subcutaneous graft of pancreas,
(*b*) with small piece of pancreas *in situ*,

(2) totally depancreatised, and

(3) under these conditions to see what effect is produced upon absorption by the addition of raw pancreas to the diet.

Methods of fat extraction and estimation.

The total quantity of faeces is dried in an air oven at room temperature, the process occupying 12–20 hours. The dried faeces are powdered thoroughly till the whole sample readily passes through a fine sieve, after which the sample is again rubbed up in a mortar till the whole is reduced to a very fine powder, which is then weighed. One gram is accurately weighed out and transferred to a Jena flask, 20 cc. 20 per cent. NaOH added, and the whole heated in a water-bath for three hours as recommended by Kumagawa and Suto [1908]. The warm alkaline solution is then poured into a separating funnel and acidified with HCl; the acid is added in small quantities and after

each addition the separating funnel is well shaken under cold running water. When the whole is thoroughly cooled, about 50 cc. of ether are added and the mixture shaken at short intervals for a quarter of an hour and set aside for a few minutes to allow the solutions to stratify. This shaking is repeated three times and the separating funnel is then set aside for an hour so that all the ether may rise to the surface and a distinct thin film of demarcation between the two fluids become apparent. The lower layer is then separated, leaving the precipitate in the funnel, and the ether poured into a 200 cc. flask. The precipitate is dissolved in NaOH and shaken with ether; the separated acid layer is returned to the funnel and the whole extracted with ether twice. The ether is then evaporated, the residue redissolved in anhydrous ether and filtered through a capillary filter similar to that described by Mottram [1909]. The resulting solution is again evaporated, the residue dissolved in petroleum ether, allowed to stand for half an hour, and filtered. The petroleum ether is evaporated and the residue dried at 70° for 4–5 hours till of constant weight.

Folin and Wentworth [1910] have shown that everything taken out by organic solvents is not fat, and that it is impossible to determine separately free fatty acids and soaps. The ordinary fatty acids are very weak, and the extent to which they combine with bases to form soaps in faeces is not important. The equilibrium between soaps and fatty acids is influenced by so many factors, H_2S, CO_2, NH_4, heat, etc., during the drying, that the proportion of soaps, free fatty acids and neutral fat at the end of the process bears no relation to that existing at the time of defaecation. The new method described by Folin and Wentworth for the estimation of the fatty acids has been used. The solvent used was warm benzene and the dissolved fatty acid was titrated with N/10 alcoholic soda, phenolphthalein being used as an indicator. On several occasions the mean molecular weight of the fat of the faeces was determined, both with normal and diabetic samples, and under normal conditions of intestinal activity, and it was found to approximate closely to the molecular weight of stearic acid.

The estimation included the total fat, extracted as fatty acid plus cholesterol, lecithin and unsaponifiable substances present in the stool. The fatty acid was determined by the titration described above, while the difference between the fatty acid and the total fat extracted by petroleum ether was taken as an indication of the amount of lecithin and unsaponifiable substances in the faeces.

The N was determined by the Kjeldahl method.

Animals.

The faeces for the periods were demarcated by an addition of bones to the diet. The dogs were weighed at regular intervals, the diabetic animals being weighed every day. While under observation the dogs were kept in metabolism cages. They were fed usually once a day (about 5 p.m.), no food being left in the cages over night. The food was weighed and precautions taken against wasting of the weighed amount.

Samples of the food were analysed from time to time. The following table gives the percentage analysis:

	N	Fat
Horse meat (cooked)	4·1 to 4·41	3·0
Ox pancreas	2·89	12·1
Caseinogen	15·0	—
Puppy biscuit	3·14	3·48
Suet	0·730	82·0
Milk	0·52	3·5
Ereptone	12·8	—

Operations.

All the operations were performed by Professor Starling. The dogs were given a small dose of morphia hypodermically about two hours previously and then the operation was carried out under CE anaesthesia.

In the case of Dog I where a graft of the gland was left, all the pancreatic tissue was removed from the abdominal cavity, a small portion of the tail of the gland with its blood supply intact through a pedicle of mesentery being placed under the skin of the abdominal wall. The graft was functional, secreting a clear fluid for a short period after the operation, which could slowly digest fibrin.

With regard to Dog II, which was also partially depancreatised, a small portion of the gland was left *in situ* close to the main pancreatic duct, which was ligated peripherally. After removal of this piece of pancreatic tissue, the dog, which had a reduced sugar tolerance, became markedly diabetic, but remained in good condition for some time.

The Utilisation of Protein N and Fat in Normal Dogs.

Table I gives the total and percentage excretion of N and fat and the percentage of fatty acid of total petroleum ether extract, which latter includes lecithin, cholesterol and unsaponifiable substances, etc.

TABLE I.

Normal Dogs.

Date		Dry wt. of Faeces g.	Total N	% N	Total fat	% fat	Total fatty acid	% fatty acid of total P. E. Extract
			1. Diet: puppy biscuit, 200 g.—5 days.					
Dec.	1	15	1·08	4·73	0·720	3·60	—	—
	3	20	0·870	4·62	1·038	7·42	—	—
			Weight, 10·15 kilos.					
			2. Diet: meat, 250 g.—5 days.					
	10	15	0·693	4·48	2·01	13·04	1·260	60
	11	20	0·574	2·87	1·59	7·98	0·963	60·5
	12	14	0·300	2·10	0·670	4·78	0·371	56·7
	14	16	0·493	3·08	0·592	3·20	0·273	53·6
			Weight, 9·70 to 9·65 kilos.					
			3. Diet: milk, 200 c.c.; puppy biscuit, 100 g.; meat, 100 g.—9 days.					
	18	17	1·05	6·23	1·67	9·80	0·91	54
	19	20	1·26	6·30	1·432	7·40	0·624	43·6
	20	23	1·16	5·04	1·45	7·80	0·759	52·1
	22	15·5	0·90	5·46	1·55	10·0	0·924	59·6
	24	24	1·377	5·74	1·69	7·0	0·824	48·8
			Weight, 10 to 9·6 kilos.					
			4. Diet: milk, c.c. per diem, 700, 700, 650, 600, 350, 250, 400, 400, 400. Puppy biscuit, 89 g. per diem (7th and 8th); 90 g. for last 3 days (9th, 10th and 11th).					
Jan.	6	23	1·03	4·48	6·716	29·2	4·301	64·2
	8	18·4	0·773	4·06	1·884	10·24	0·889	50·2
	9	19·5	1·133	5·74	1·840	9·46	0·866	47·8
	12	30	1·470	4·76	1·530	5·14	0·766	56·8
			Weight, 10 to 10·1 kilos.					
			5. Diet: milk, 400 c.c.; puppy biscuit, 90 g.; lemco, 4, 8, 12, 16, 30, 16, 16, 8 g. per day.					
Feb.	15	37·5	1·84	4·9	1·77	5·55	1·065	51·4
	17	22·5	1·230	5·45	1·07	4·35	0·639	65·1
	20	42·0	2·226	5·32	2·788	6·64	0·984	40·0
			Weight, 8·85 to 8·80 kilos.					
			6. Diet: meat, 400 g. first 2 days; 750 g. next 3 days; ereptone, 134·5 g.					
Mar.	4	33·3	1·84	5·53	2·84	8·86	1·230	45·0
	7	32·0	2·24	7·28	3·2	9·92	1·635	51·0
	9	20·6	1·241	6·02	2·04	9·90	1·179	54·5
			Weight, 9·10 to 9·00 kilos.					
			7. Diet: meat, 143 g.; suet, 72 g. per diem (average)—13 days.					
Jan.	18	27·2	1·104	4·06	3·56	13·16	2·086	58·3
	22	26·0	1·40	5·67	8·03	30·8	5·096	63·4
	25	20·0	1·176	5·88	5·08	28·8	3·692	64·0
	30	26·0	1·456	5·60	6·55	25·2	3·640	57·0
			Weight, 9·9 to 9·00 kilos.					

TABLE I—*Continued*

Date	Dry wt. of Faeces g.	Total N	% N	Total fat	% fat	Total fatty acid	% fatty acid of total P. E. Extract
8. Diet: cascinogen, 60 g.; palmine, 30 g. per diem for 7 days.							
Mar. 11	16·5	0·880	5·32	1·73	10·1	1·218	73·1
13	12·1	0·545	4·55	2·34	19·3	1·224	52·8
14	10·3	0·462	4·48	1·09	10·56	0·789	74·0
15	11·0	0·531	4·83	0·891	8·10	0·625	70·2
16	10·65	0·522	4·90	0·880	8·12	0·667	77·7
18	21·6	0·937	4·34	2·268	11·08	1·944	86·0
Weight, 8·90 to 8·75 kilos.							
9. Diet: cascinogen, 60 g.; cod liver oil, 40 c.c. (17th–26th).							
Mar. 19	11·6	0·544	4·90	0·673	6·06	0·395	58·6
21	9·7	0·489	5·04	0·440	4·46	0·275	61·6
23	12·0	0·554	4·62	0·430	3·58	0·307	71·5
24	13·8	0·656	4·76	0·620	4·52	0·391	63·1
25	9·8	0·384	3·92	0·637	6·78	0·445	69·9
26	15·3	0·686	4·48	0·921	6·32	0·782	25·0
29	15·0	0·609	4·06	1·65	11·08	0·879	53·3
27th and 28th—Butter fat, 40 g. per diem. Weight, 8·75 to 8·78 kilos.							

Utilisation of N.

No. 1 shows results upon an exclusive diet of 200 g. of puppy biscuit, given over a period of five days; the utilisation of the N is 93·8 per cent. of the total ingested. On a diet of cooked meat the total N excreted falls, and as the results in No. 2 show, the protein of meat is readily dealt with by the normal gastric and intestinal secretions, only 3·5 per cent. being lost by the faeces. On a mixed diet of meat, milk and puppy biscuit (No. 3), the total daily excretion of faecal N rises and remains steady at about 1 gram. The absorption figure of 89·71 per cent. is probably lower because of the large amount of starch in puppy biscuit which is available for the immediate supply of energy.

No. 4 shows the effect of a milk diet continued for a period of nine days. During the first two days 700 cc. of milk were ingested, and on the third day of the period the first estimation of N gives a remarkably uniform figure. In the course of the last six days of the experiment 2400 cc. of milk and 448 g. of puppy biscuit were given, equal to 25·44 g. N, of which 3·376 g. were found in the faeces, giving an absorption of 92·70 per cent. For the whole period the utilisation figure for N was 87·2 per cent.

In No. 5, where the diet consisted of 400 cc. of milk with puppy biscuit and lemco, the latter in quantities of from 2–30 g. per diem, the percentage

N excreted daily is not appreciably altered, but here the utilisation is very good, being 97·27 per cent. This of course may be due to the appetising effect of the added lemco; at any rate it shows that given a good appetite and milk not in excess of the needs of the organism, the protein, fat and carbohydrate of milk will be practically all assimilated.

The protein of milk is well utilised and even the addition to the diet of concentrated protein preparations does not appreciably alter the daily loss of N. The results, as shown in No. 6, where ereptone was added to a diet of meat sufficient in itself, clearly demonstrate the avidity and the ease with which the organism deals with protein. A loss of only 5·28 g. of N on such a nitrogenous diet is remarkable.

A diet of meat and mutton fat given over a period of nineteen days resulted in a slight increase in the dry bulk of the faeces which could be accounted for by the nature of the diet and the longer period between the days of defaecation. As No. 7 indicates, the daily N is slightly increased, but the percentage is within the usual normal variations. The percentage utilisation of N is 96·2.

On a diet of caseinogen and palmine (No. 8) the absorption of N is 95·37 per cent. The addition of cod liver oil to the diet had practically no effect upon N absorption, the percentage N utilisation in No. 9 being 96·5.

The N *of the faeces.*

It is known that the composition of the faeces is not greatly altered by feeding dogs on any of the simple food stuffs. This does not hold good for herbivora. Fritz Voit [1892] by his loop experiments showed that the excretions from the isolated gut were similar in constitution to that obtaining in the whole gut. The N content was almost equal in both as calculated per square metre per 24 hours. The loop however contained a greater amount of fat and fatty acids. From these experiments he maintained that the N of the faeces was largely derived from substances excreted through the wall of the intestine and was therefore a product of protein metabolism.

In my tables it is seen that the N excretion remains fairly constant on any diet which does not consist exclusively of meat, for on such a pure meat diet the N excretion is reduced from the approximate daily loss of 1 g. to 0·5 g. per day, the utilisation varying from 89·71 per cent. to 96·53 per cent.

With milk the N figure averages 1·1 g. per day, but the addition of carbohydrate raises the daily N loss to 1·5 g. per day. Where vegetables

are added to a large milk diet the N loss is evident, but whether this increase is due to an increased secretory activity or less complete utilisation of N, it is difficult to say. As has been pointed out in Nos. 4 and 5, milk, when given in normal amounts, is best assimilated when such a palatable article of diet as puppy biscuit is added. As with meat so with all concentrated protein foods, the N loss is small, the percentage daily loss varying from 3·50 to 5·28 g., which is equivalent to a total daily loss of from 0·38 to 0·71 g. On a low N diet the utilisation was 88 per cent., while on a high N diet the utilisation averaged 95·89 per cent.

Utilisation of fat.

On a mixed diet the fat absorption varies from 89 to 96 per cent., and depends upon the form in which the fat is ingested. On a diet of puppy biscuit the fat absorption was 94·98 per cent., while on a diet of meat only (No. 2) the absorption was 87·1 per cent. In this latter case the fatty acids recovered from the faeces amounted to 7·6 per cent. of total fat in diet, which is equal to a percentage utilisation of pure fat, estimated as fatty acid, of 92·4. An increase of fat in the diet leads to an increased percentage utilisation, as shown by the results in No. 3, where the absorption figures for fat as total petroleum ether extract and pure fat are 92·98 per cent. and 95·86 per cent. respectively.

In No. 4, where the diet consisted largely of milk, 87·68 g. of fat were given during the last six days of the experiment, of which 5·254 g. were lost, which is equal to a utilisation of 94·0 per cent. For the total period of the experiment the absorption of total fat amounted to 91·82 per cent. and of pure fat to 95·34 per cent., proving as has been done so often that a natural emulsion of fat such as obtains in milk is the best condition for its assimilation. On a mixed diet of milk, puppy biscuit and lemco excellent utilisation figures were obtained, as the results in No. 5 show. The amount of fatty acid recovered from the faeces was 2 per cent. of the total fat given, while the total fat extracted amounted to 3·64 per cent. of the fat ingested.

Mutton fat, which has a melting point of from 49° to 51°, is well utilised even when given in large quantities of 50 to 90 g. a day. This confirms von Noorden's statement that a larger quota of fat in the diet effects a more complete absorption. Here 807·86 g. of fat were given during the period under observation; the meat in the diet accounted for 85·6 g. of fat, making a total of 893·46 g. of fat, of which 23·94 g. were excreted, giving a utilisation of 97·4 per cent. The fatty acid recovered from the faeces amounted to

1·79 g., which is equivalent to a pure fat utilisation of 98·21 per cent. Munk [1884], feeding dogs with suet alone, obtained results indicating an absorption of 90 to 94 per cent. of fat.

Stearin and palmitin are the chief constituents of several preparations which are made to take the place of butter, such as margarine and palmine, etc. The stearin of these preparations is extracted from "kernel" paim nut oil after the removal of the more liquid portion by hydraulic pressure. The melting point of the palm nut oil is 34°–40°, its chief constituent being palmitin. The small amount of stearin in palmine gives to it that solid consistency which makes it such an ideal preparation for feeding purposes.

During the experiment—No. 8—204·0 g. palmine were ingested and 9·199 g. of fat excreted, which is an absorption of 95·5 per cent.; the pure fat utilisation was 95·6 per cent.

No. 9 shows that the free fatty acid in cod liver oil facilitates the emulsifying and the subsequent absorption of the oil; 360 g. of the oil were given and 3·721 g. of fat and fatty acid were excreted; utilisation, 98·97 per cent. A point of note here is that the percentage fatty acid of total fat extracted is distinctly higher than in the preceding tables, and estimating the utilisation from the point of total fatty acid recovered we find almost a total assimilation, 99·04 per cent.

On a low fat diet the utilisation was 87·1 per cent. and on a high fat diet 97·4–98·9 per cent.

The effect of pancreas feeding.

It could not be expected that the addition of pancreas to the diet in normal dogs would materially improve such absorption figures as 97·27 per cent., etc., which are the result of feeding on a mixed diet composed largely of meat, with the addition of puppy biscuit and a bone, ingredients calculated to bring into play to their fullest extent not only the physical but the psychical factors in digestion.

A dog fed on 250 g. of meat and 150 g. of pancreas for three days excreted at the end of that period 1·84 g. N (5·74 per cent.) and 1·92 g. fat (6·25 per cent.), which is equal to a utilisation of N, 96·15 per cent. and of fat, 97·15 per cent.

Utilisation of Protein N and Fat in Diabetic Animals.

A. *In partially depancreatised dogs.*

Condition of animals. The day after operation Dog I, which had a graft of pancreatic gland in its abdominal wall, weighed 8·6 kilos and excreted

1·85 g. sugar in the 24 hours sample of urine. On the following day the loss of sugar had risen to 30 g., about which figure it remained for a week, after which for a few days the excretion rose to between 40 and 50 g., subsequently falling to 28 g. and remaining with varying fluctuations at about 30 g. per day till the eighteenth day after operation, upon which day the dog was killed by bleeding under $CHCl_3$ anaesthesia. The D : N ratio in this dog varied from 3·8 : 1 on the third day to 5·4 : 1, which latter figure was reached on the tenth day after operation, the ratio for the following days being between 3·2 : 1 and 5·0 : 1. From these data one sees that despite the graft of pancreatic tissue and the apparently good condition of the animal, a decidedly diabetic condition obtained, in view of which the following figures for utilisation are worthy of note. In comparison with the totally depancreatised dogs the slower loss in weight and the higher respiratory quotient—0·69 to 0·740—show that the disturbance was not so acute as in cases of immediate depancreatisation.

Dog II, in which a portion of the gland was left *in situ*, weighed 8·0 kilos before operation. On the fourth day after operation the sugar excretion had risen to 5 g. in the 24 hours with a D : N ratio of 1 : 0·7, while on the eighth day the total excretion for the 24 hours was 11·0 g., from which time it rapidly fell until only a trace of sugar upon occasional days could be obtained. The loss of weight was very gradual, the respiratory quotient remained normal 0·78 to 1·03 according to diet, and the glycosuria, up to the time the dog was totally depancreatised, was transient.

Utilisation of N. Dog I. In Nos. 10 and 11 (Table II) the N absorption is 93·2 and 95·4 per cent. Pancreas was added to the diet in both cases;

TABLE II.

Partially depancreatised Dogs.

10. Dog I. Partially depancreatised; c graft in abd. wall.

Diet: May 21, meat 100 g.; May 22–June 3, meat, 150 g., pancreas, 50 g.; June 3–8, meat, 200 g., pancreas, 50 g.; June 9 and 10, meat, 200 g., pancreas, 60 g.

Date	Dry wt. of Faeces g.	Total N	% N	Total fat	% fat	Total fatty acid	% fatty acid of total P. E. Extract	Wt. of dog in kilos
May 23	44·0	2·834	6·44	13·53	30·76	10·34	76·6	8·1
26	14·1	0·852	6·04	4·935	33·76	3·567	70·47	7·60
June 2	35·0	2·264	6·44	10·50	30·10	7·154	72·7	6·80
6	27·0	1·890	7·0	4·320	16·56	3·604	80·9	6·60
7	26·0	1·929	7·42	3·640	14·0	2·496	69·0	6·50
11	24·2	1·829	7·56	2·90	12·16	1·920	66·2	6·20

Weight before op. 9·15 kilos. Op. 18th. Weight 20th, 8·65 kilos.

TABLE II—*Continued.*

11. Diet: caseinogen, 60 g.; pancreas, 100 g.—19 days.

Date		Dry wt. of Faeces g.	Total N	% N	Total fat	% fat	Total fatty acid	% fatty acid of total P. E. Extract	Wt. of dog in kilos
June	15	17·7	0·977	5·18	1·848	10·44	1·358	73·4	6·0
	16	23·4	1·579	6·72	0·799	3·36	—	—	5·75
	17	24·0	1·310	5·46	2·112	8·88	1·632	77·2	5·70
	19	21·4	1·02	4·76	1·914	8·94	1·519	79·4	5·60
	20	28·2	1·421	5·04	2·989	9·68	1·762	61·9	5·50
	22	36·4	1·783	4·90	4·659	12·20	3·101	69·8	5·45
	23	8·0	0·3584	4·48	1·280	15·76	1·20	94·0	5·25
	26	23·3	0·9786	4·20	6·291	26·82	5·545	87·7	5·10

12. Dog II. Portion of gland left *in situ*; c pancreatic duct intact.
Diet: meat, 100 g.; pancreas, 50 g.; May 21–June 3. Meat, 150 g.; pancreas, 50 g.; June 4–10.
Weight, 8·0 kilos. Op. 18th. Weight, 20th, 7·75 kilos.

May	22	30·5	0·818	9·24	5·155	16·98	4·575	85·1	7·40
	24	12·4	1·160	9·36	1·860	15·5	1·50	80·7	7·20
	26	27·0	2·419	8·96	5·94	22·0	—	—	7·00
	27	40·0	3·080	7·70	4·40	11·4	3·290	74·8	6·90
	28	9·0	0·680	7·56	1·681	18·68	1·07	71·0	6·80
	31	8·5	0·643	7·56	1·275	15·12	1·013	78·8	6·80
June	1	35·0	2·264	6·44	10·5	28·12	7·154	72·7	6·75
	2	5·6	0·450	7·98	0·622	11·1	0·397	65·0	6·75
	3	9·2	0·834	6·44	1·454	15·1	1·097	75·4	6·75
	4	11·5	0·744	6·86	1·242	10·88	0·654	53·0	6·85
	6	16·8	1·15	8·40	1·405	8·36	0·814	60·3	7·00
	8	11·0	0·924	7·70	1·430	12·381	0·782	59·4	6·75
	10	20·5	1·578	8·96	3·895	18·68	2·844	70·9	—
	11	12·6	1·130	7·98	1·385	10·94	0·894	71·0	6·75

13. Diet: meat, 150 g.; pancreas, 30 g. (16th to 20th).

17	8·5	0·607	7·14	0·822	9·66	0·434	55·5	6·75
18	13·55	1·021	7·564	1·49	10·90	1·035	66·7	—
20	11·3	0·886	7·84	1·34	11·04	0·7063	53·0	6·75

14. Diet: meat, 250 g.; pancreas, 50 g.—5 days.

13	21·5	1·535	7·14	3·775	17·56	1·587	42·0	6·35
14	18·0	1·285	7·14	2·660	14·80	1·89	74·6	6·40
16	16·7	1·403	8·40	2·348	14·06	0·902	40·0	6·50

15. Diet: June 30, meat, 100 g., puppy biscuit, 100 g.
Thyroid, July 2–4, 1 g.; 5–6, 1·5 g.; 7–8, 2 g.; 9–10, 3 g.

July	1	—	0·916	—	0·891	—	0·810	61·4	—
	2	23·6	1·784	7·56	2·025	8·58	1·376	68·2	6·60
	3	17·0	1·142	6·72	0·8262	4·86	0·435	52·6	6·60
	4	30·0	2·058	6·86	1·764	5·88	0·936	55·0	—
	5	31·2	2·184	7·0	1·50	4·84	0·775	52·8	6·62
	6	17·0	1·071	6·3	1·221	7·10	0·675	56·8	—
	7	26·0	1·310	5·04	1·70	6·54	1.107	65·1	6·65
	8	39·2	2·566	6·58	2·74	7·0	1·113	41·0	6·60
	9	32·5	2·138	6·58	2·35	7·20	1·170	50·0	6·50
	10	24·7	1·729	7·0	2·021	8·18	1·052	52·1	—
	11	35·8	1·356	6·30	2·899	8·10	1·525	52·6	6·40

in the former the chief constituent was meat, 150–200 g. per day, in the latter, caseinogen, 60 g. per day. Both these experiments show a remarkable utilisation of N, the protein being presented in a most assimilable form.

Dog II. The figures in No. 12 are the result of a diet similar to, but more generous than, the preceding, and the observation was continued over a much longer period—three weeks. The excretion of N is less; during the period 2490 g. of meat and 890 g. of pancreas were given, which is equal to 102·09 g. and 26·7 g. N respectively, making a total of 128·79 g. N, of which 19·87 g. were excreted, giving a utilisation of 86·0 per cent. The average daily excretion, which was 0·990 g. N, is quite a normal figure.

In No. 13, where the same diet was repeated, there was an improved N utilisation, 91·7 per cent. An increased meat diet with pancreas still maintains an improved absorption, as is shown by No. 14, where the N utilisation was 93·27 per cent.

These high figures can be due to no other cause than the beneficial effect exerted by the added pancreas in the diets, because on a mixed diet of meat and puppy biscuit, which in normal animals leads to improved protein absorption, and regardless of the fact that the protein need of the organism was accentuated by thyroid administered, the digestive system did not on this mixed diet, without pancreas, maintain its previous powers of utilisation, the N absorption falling to 79·86 per cent. (No. 15.)

It is seen that where a portion of the gland is left *in situ* the results are similar to those obtained with a graft of the gland. Most of the previous work has been done irrespective of the addition of pancreas, and in these experiments where no pancreas has been added to the diet, the N loss has varied from 17 to 27·5 per cent.

Utilisation of fat. In Dog I the amount of fat ingested was 209 g. and that excreted 39·82 g., which gives a utilisation of 80·95 and of pure fat 86·1 per cent. The first sample of the experiment contained a large amount of fat, 13·53 g. The second, three days after, which was of much smaller bulk, contained 4·935 g. The percentage of fat in both samples was over 30. These figures are high because of the constipation existing, but with a more regular action of the intestine the total amount of fat per sample approximates more closely to a normal figure, but that fat is being lost is quite evident both from the total and percentage figures. In No. 12 almost the same amount of fat was given, there being double the daily amount of pancreas ingested as in the previous case. Here the absorption figure has improved, amounting to 87·44 per cent. of total fat and 91·7 per cent. of pure

fat, calculated as fatty acid. While it is possible to get a good absorption of fat, it cannot be maintained for long periods; as can be readily understood, a diabetic organism cannot be expected to show the same adaptability as the normal.

Dog II was fed for three weeks on a meat and pancreas diet. The fat loss was comparatively large, being 23·9 per cent., a figure which is more in agreement with Weintraud's results, but in view of better absorption on other occasions cannot be accepted as being an indication of the failure of the diabetic to absorb fat to any great extent.

On a rather sparing diet of meat and pancreas the amount of fat given was 30 g., of which 3·65 g. were excreted, being equal to a utilisation of 87·83 per cent. estimated as total fat absorbed and of 92·75 per cent. as pure fat. With a more liberal diet similar to the foregoing the utilisation was practically the same, viz. 85·95 per cent. and 93 per cent. (No. 14.)

With partially depancreatised dogs on a diet of meat and pancreas there is an average loss of fat amounting to 14·37 per cent.

On a diet of meat and puppy biscuit without the addition of pancreas, the utilisation of the small amount of fat in the diet is very poor (No. 15), the figures being, for total fat, 72·12 per cent., and for pure fat, 84·7 per cent. A loss of 27·8 per cent. of fat on such a diet should never occur when pancreas is added. In all these cases of partial depancreatisation the excretion of fatty acid with pancreas feeding is 60–80 per cent. of the total petroleum ether extract. The lack of pancreas in the diet results in a lowering of the percentage excretion of the fatty acid to between 40 and 65.

B. *In totally depancreatised dogs.*

Condition of the animals. As several dogs having a graft of pancreas in their abdominal wall or a piece left *in situ* have been kept in this laboratory in good condition for over two months, this preliminary operation, leading to a mild type of diabetes, undoubtedly allows the animal to adapt itself to the new conditions and so lessens the shock of the subsequent total extirpation; it also helps the animal to conserve its energy for a longer time during the much more severe type of diabetes produced by the second operation.

In totally depancreatised dogs there is always some evidence which at first hand leads one to suspect an increased amount of fat in the faeces. On a diet of meat there is an increased faecal odour, due to protein decomposition in the large intestine, and with a diet of fat there is invariably a typically

fatty foul-smelling stool, which after drying on glass plates presents the appearance of sheets of dark brown gelatin. It is evident therefore that the digestive and absorptive powers will vary according as the dog has been totally depancreatised by two stages or at one operation, because of the sudden onset of severe diabetes and the worse condition of the dog after immediate depancreatisation.

TABLE III.

Totally depancreatised Dogs.

16. Dog III. Total depancreatisation.

Diet: July 19, meat, 100 g., pancreas, 50 g.; July 22, meat, 200 g., pancreas, 100 g.

Date		Dry wt. of Faeces g.	Total N	% N	Total fat	% fat	Total fatty acid	% fatty acid of total P. E. Extract	Weight of dog in kilos
July	21	14·6	1·242	7·70	4·322	29·6	2·073	48·0	5·80
	22	15·6	1·267	8·12	2·833	18·16	2·037	71·9	5·80
	23	9·3	0·890	9·52	1·55	16·66	1·109	71·6	5·70
	24	11·7	0·982	8·40	2·14	18·28	1·183	55·0	5·65
	25–26	28·1	2·469	8·82	4·158	14·80	2·154	57·8	5·60
	27	10·3	0·894	8·68	1·456	14·14	0·731	50·2	5·50
	28	11·1	0·8081	7·28	2·775	25·0	1·607	57·9	5·35
	30	14·6	0·930	6·44	3·35	23·10	1·989	59·0	5·20

Before op. 6·5 kilos. Op. July 17. Weight, July 19, 6·15 kilos.

17. Dog IV. Totally depancreatised.

Diet: Feb. 1, meat, 500 g. for over 3 days.

Feb.	3	34·5	3·236	9·38	8·280	23·90	7·445	89·6	6·8
	4	16·5	1·60	9·94	3·30	20·44	2·673	81·0	5·6

Op. Jan. 30; weight before, 7·0 kilos; Feb. 1, 6·35 kilos.

18. Diet: meat, 160 g.; pancreas, 50 g.; milk, 50 g.—5 days.

21	18·0	1·323	7·35	3·240	18·46	2·45	75·7	6·75
22	11·2	0·909	8·12	1·789	17·10	1·590	83·0	6·50
23	14·6	1·267	8·68	1·752	12·051	1·452	80·0	6·40

After op. 16th, 7·2 kilos.

19. Diet: meat, 600 g.; pancreas, 100 g. (i.e. total).

Mar.	5	12·6	1·12	9·73	2·06	15·37	1·431	70·0	7·6
	6	24·4	1·133	4·76	4·88	19·8	3·618	74·1	7·3
	7	24·8	2·15	8·68	4·037	16·28	2·536	63·5	6·7

Op. Mar. 2. Weight before op. 8·0 kilos; after Mar. 4, 7·5 kilos.

Dog III from which the results in No. 16 (Table III) were obtained was made totally diabetic by the two-stage operation, and a marked glycosuria immediately supervened upon the second operation, the dog eliminating 80 to 100 per cent. of any sugar given to it. The D : N ratio varied between 2·8 and 3·1 : 1, and the total excretion of sugar for 24 hours was between

30 and 40 g. The loss of weight was at about the rate of one kilo in ten days, which is similar to what obtained in the first partially depancreatised dog. Another sign that a distinct metabolic disturbance had ensued was the low level of the respiratory quotient, the rise of which consequent upon sugar injection was greatly diminished. Dog IV was the subject of severe diabetes, losing weight rapidly and excreting large quantities of sugar. This dog as well as the two following (Nos. 18 and 19) were totally depancreatised at one operation, and, as will be pointed out later, received no pancreas in their diet. The others, though not wasting so rapidly as the former, lost flesh somewhat more quickly than those dogs which were only partially depancreatised. They had a D : N ratio of 3·8 : 1 and upwards, excreted 15 to 50 g. of sugar per diem, and eliminated 85–100 per cent. of sugar added to their diet.

Utilisation of N. The results obtained from a dog which had its pancreas removed in a two-stage operation are given in No. 16. The diet consisted of meat and pancreas and was in excess of the absolute requirements of the organism. The N utilisation here was 92·1 per cent., which is even within the limits of the absorption of normal animals. The following experiment shows very clearly what a detrimental effect immediate depancreatisation has upon the digestive functions of an animal. No pancreas was added to the diet. There was a loss of 21·9 per cent. N given in the food. In contrast to this, No. 18 shows how effective is the addition of pancreas. Over a period of five days 44·0 g. N were given, of which 3·499 g. were recovered in the faeces, which amounted to a utilisation of 92·0 per cent.

It is evident that for good absorption the dog must make a good recovery after the operation. The absorption shown in No. 19 is very poor, due doubtless to the fact that the dog was not in good condition, for this dog showed signs of gastric and intestinal disturbances on the fourth day after operation and died suddenly on the evening of the fifth. The amount of protein ingested as meat was approximately 500 g. and 100 g. of pancreas, equal to 25·05 g. N. 4·413 g. were excreted, giving a utilisation of 82·43 per cent.

Utilisation of fat. The absorption of fat in a dog made diabetic by the two-stage operation was very good, falling very little below the best obtained with a graft of the pancreatic gland. The amount of pure fat recovered from the faeces in this case was 10·06 g., which gives a percentage absorption of 89·94, while for total fat estimated the utilisation figure was 82·7 per cent.

The results of the following experiment (No. 17) are typical of the

far-reaching disturbance of fat digestion and assimilation consequent upon immediate total depancreatisation, when no pancreas is administered with the food. The total fat recovered was 67·4 per cent. of the amount given, being equal to 32·6 per cent. utilisation.

That pancreas influences fat assimilation to a marked degree is shown by the results in No. 18, where the utilisation figures for total fat and pure fat were 88·3 per cent. and 90·5 per cent. respectively. Once digestive disturbances have set in, all absorption will become deranged, no matter what dietetic means be taken to improve it, e.g. the addition of puppy biscuit or pancreas: this is shown by the poor fat absorption figure of 69·8 per cent. (No. 19.)

Discussion.

These results indicate that the removal of the pancreas causes an immediate and serious disturbance of the digestive functions as regards both protein and fat, and also, as the conditions of these animals show, the power of oxidising glucose is more or less destroyed according to the degree of the diabetes produced.

We see from these results that the figures for utilisation in cases of total depancreatisation with pancreas feeding are somewhat better than in those of partial depancreatisation without pancreas. Still in the total diabetic the function of the absent pancreatic juice must be carried on by other secretions, gastric and succus entericus, as we have no knowledge of any deterrent effect upon the secretions of other juices. Moorhouse [1915] in a recent publication shows that in partially depancreatised dogs there may be an increase of metabolism amounting to 9 per cent., while in total depancreatisation such an increase may vary from 9 to 38 per cent. of the normal. He holds that this increase of metabolism runs parallel to the severity of the case and that it is an expression primarily of interference with carbohydrate utilisation and secondarily with protein and perhaps fat metabolism. The protein destruction is enormously increased, while the actual part taken by protein in the oxidations, i.e. the energy distribution of the proteins, is only slightly increased, 5 to 10 per cent. This increased metabolism would explain the good percentage utilisation of protein and fat in the attempt to make good the great expenditure, when the power of sugar utilisation is more or less lost.

Conclusions.

1. In normal dogs the N utilisation on a high protein diet varied from 95 to 96·5 per cent., while on a low protein diet it was 88 per cent.

2. Fat utilisation on a high fat diet was 97 to 98·9 per cent., on a low diet 87·1 per cent.

3. In partially depancreatised animals there is very little difference in power of assimilation, whether the piece of gland is grafted in the abdominal wall or left *in situ*, but the addition of pancreas to the diet exerts a most favourable effect in both cases.

Utilisation figures:

With part of gland grafted ..	N = 93–95·4 %	With pancreas	
	Fat = 80–87·4 %		
With part of gland *in situ* ..	N = 86–93·27 %		
	Fat = 76–88 %		
Without pancreas	N = 79·86 %		
	Fat = 72·12 %		

4. Total depancreatisation leads to an immediate and severe diabetes which is usually terminal in about a week or ten days. This condition is alleviated by performing the operation in two stages, the first being a partial depancreatisation.

5. The addition of pancreas to the food is as beneficial in one-stage operations as it is in the two-stage operation.

6. Immediate depancreatisation without pancreas in the diet produces grave disturbances of digestion and absorption, resulting in a loss of 22 per cent. of N and 67·4 per cent. of fat. The addition of pancreas is most beneficial, the utilisation of N rising to 92 per cent. and of fat to 70–88 per cent.

7. The deleterious effects of extirpation of the pancreas on fat absorption and nitrogen metabolism are thus by no means so serious as has been found by other observers quoted in the introduction to this paper.

I am greatly indebted to Professor Starling for performing the various operations upon these dogs, and for his advice and interest in the work.

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XV. A NEW TEST FOR REDUCING SUGARS IN URINE.

By WILLIAM CRAMER.

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The test depends upon the reduction of mercuric oxide in a weakly alkaline solution to metallic mercury. The degree of alkalinity is an important factor, as the test becomes more sensitive but less specific the greater the alkalinity of the reagent. The reagent is prepared as follows: 0·4 g. mercuric oxide (red or yellow) and 6 g. potassium iodide are dissolved in 100 cc. water. This solution is weakly alkaline. The alkalinity must now be so adjusted that 10 cc. of the reagent are neutralised by 2·5 cc. of n/10 acid, using phenolphthalein as an indicator. This is done by titrating 10 cc. of the reagent with n/10 acid and, after the alkalinity of the reagent has thus been determined, adding the requisite amount of n/10 acid or alkali to the bulk of the reagent. The reagent is a clear, colourless solution which turns slightly yellow on heating and becomes colourless again on cooling. It must remain quite clear on boiling. The reagent thus prepared will be referred to in this paper as the "2·5 standard reagent" in order to indicate its alkalinity.

Potassium cyanide may be used as a solvent for mercuric oxide, but potassium iodide has been found to be preferable, and all the data given in this paper refer to the reagent prepared with potassium iodide.

With this reagent the test if applied to an aqueous solution of a sugar is carried out as follows: 3 cc. of the reagent are heated in a test-tube to boiling. The solution remains clear, but turns slightly yellow. 0·3 cc. of the sugar solution is now added and the mixture again heated to boiling. The test-tube is then removed from the flame. The mixture darkens and a black precipitate of finely divided mercury appears. At first this precipitate gives the appearance of a turbidity, especially if the amount of sugar present is small. On standing, the precipitate soon settles at the bottom of the test-tube.

The test is positive with sugars which give the other reduction tests such as lactose, maltose, xylose and arabinose. It is negative with cane sugar.

An indication of the sensitiveness of the test is given by the fact that a slight reduction can still be obtained with one milligram of glucose if an aqueous solution of glucose is used.

If the test is applied to normal urine in the way to be described below, a slight but distinct reduction is obtained comparable in degree to that obtained with aqueous solutions of glucose containing 0·1 per cent. to 0·2 per cent. of glucose.

The alkalinity of the reagent has been fixed at the standard given above for the following reasons. The greater the alkalinity of the reagent the greater is the degree of a reduction produced by a given amount of a reducing sugar, so that the reagent could be made more sensitive by increasing its alkalinity. If, however, the reagent is made strongly alkaline, it ceases to be specific for reducing sugars and chemically allied substances, and is reduced by other organic substances, such as creatinine. And since sugars have to be identified as a rule in fluids such as urine and serum containing other organic substances, the reduction of the reagent by substances other than reducing sugars and chemically allied substances must be excluded, if the test is to be an improvement on Fehling's test, where the reduction by substances such as creatinine and uric acid constitutes a serious fallacy, particularly if small amounts of sugar are being tested for.

The alkalinity of the reagent was fixed at the 2·5 standard because at that point the reduction produced by normal urine is very slight and corresponds in degree to that produced by aqueous solutions containing 0·1 per cent. to 0·2 per cent. of glucose. It is then certain that the reduction produced by normal urine is due only to the glucose which it contains and not to any other constituent. This was confirmed by testing the reagent with a number of organic substances, present normally, or likely to be present, in urine, such as urea, uric acid, urates, guanine, allantoin, creatinine, hippuric acid, taurine, bile salts. In amounts exceeding those possibly present in urine all these substances gave negative results.

When the alkalinity of the reagent is increased so that 10 cc. of the reagent are neutralised by 3 cc. n/10 acid ("3·0 standard reagent"), normal urine gives a reduction slightly greater than that produced by 0·1 per cent. to 0·2 per cent. solutions of glucose. It does not necessarily follow that this greater reduction is due to some normal urinary constituent other than glucose. For the 3·0 standard reagent shows no reduction when tested with the urinary constituents mentioned above in concentrations exceeding those found in normal urine. The observations recorded in a previous paper [Cramer, 1915] suggest the possibility that, just as in the case of cupric

oxide, urine presents conditions more favourable for the reduction of mercuric oxide by glucose than a pure aqueous solution. But in order to exclude possible fallacies the 2·5 standard reagent is recommended and not the slightly more sensitive 3·0 standard reagent.

If, on the other hand, the alkalinity is reduced so that 10 cc. of the reagent are neutralised by 2 cc. n/10 acid ("2·0 standard reagent"), normal urine gives practically negative results, and a distinctly positive result is obtained only if the urine contains as much as about 0·3 per cent. of glucose. An example of the differences obtained with the reagents of different degrees of alkalinity is given in Table I.

TABLE I.

Glucose-solution	"2·0 Standard"	"2·5 Standard"	"3·0 Standard"
0·1 %	hardly visible	very slight	slight
0·2 %	slight	slight	slight
0·5 %	distinct	marked	intense
1·0 %	intense	intense	intense
Urine containing glucose			
0·1 % (normal urine)	hardly visible	very slight	distinct
0·2 %	hardly visible	slight	distinct
0·3 %	distinct	distinct	intense
0·5 %	distinct	intense	intense

There is, of course, no difficulty in recognising the presence of large amounts of reducing sugars by this reagent whether the "2·0 standard," the "2·5 standard" or the "3·0 standard" is used. But the value of this reagent lies in the fact that it enables one to recognise readily very small quantities of sugar in urine, when Fehling's test gives only doubtful results, while Nylander's test is less sensitive and requires for the identification of small amounts prolonged boiling which is often troublesome. A comparison of

TABLE II.

Specimen of urine	Glucose %	Fehling	Nylander	"2·0 Standard"	"2·5 Standard"	"3·0 Standard"
M	0·07	negative (transparent green)	negative	very slight	distinct	distinct
C 1	0·2	doubtful (opaque greenish brown)	doubtful	hardly visible	distinct	distinct
C 2	not determined	probably positive (transparent brown)	distinctly positive	distinct	distinct	distinct

the different tests is given in Table II. The tests were made with three different specimens of urine from two normal individuals. The amount of glucose present was determined by Bertrand's method, using the modification devised by MacLean and Gardiner [1914] for small quantities of glucose.

As Table I shows, the test here described enables one to recognise not only the presence of small amounts of reducing sugars, but also slight differences in the amounts of sugar present, when these amounts are small. But in order to do so it is necessary to carry out the test in such a way that the conditions under which reduction is produced are constant and that the turbidity, which is a measure of the amount of reduction, is due entirely to the precipitated mercury and not to the phosphates, which are, of course, also precipitated when the urine is added to the hot alkaline reagent. The turbidity due to phosphates can be excluded simply by acidifying the mixture, after the test has been carried out, with acetic acid, which dissolves the phosphates. Since the addition of acetic acid stops the reduction which would otherwise proceed in the hot mixture, it is necessary to add the acid after a fixed interval, say 30 seconds. As further the precipitate of mercury is heavy and rapidly falls to the bottom of the test-tube, the degree of turbidity must be judged at once. In the table the degree of turbidity is described as "intense" when ordinary print cannot be read through the mixture. The terms "slight" and "distinct" are applied when print can be read with ease through the mixture. The term "marked" means that print can be read only with difficulty. This "print reading test" is a useful criterion of the degree of reduction produced by small quantities of sugar. As stated above it must be applied immediately after the addition of the acetic acid.

The test, then, as applied to urine, which must, of course, not have undergone ammoniacal fermentation, is simply as follows: 3 cc. of the 2·5 standard reagent are heated to boiling in a test-tube (of ordinary dimensions). The reagent must remain clear. 0·3 cc. of urine is added and the mixture again heated to boiling. The test-tube is removed from the flame and, after thirty seconds, the mixture is acidified with a few drops of acetic acid. The test-tube is at once held over ordinary print. If the urine is normal, a slight but distinct turbidity remains and print is clearly readable through the mixture.

It may be suggested that the "2·0 standard reagent" is more suitable for clinical use as it gives a negative result with normal urine. But there is no reason why the small amount of glucose present normally in urine should be deliberately ignored, if it can be readily recognised. Moreover, the differences in the degree of reduction given by urines containing a slight excess of glucose (0·2 per cent. to 0·5 per cent.) are better marked with the "2·5 standard reagent" than with the less sensitive "2·0 standard reagent."

The reduction of mercuric salts by sugar in alkaline solution has been used before by Knapp [1870] as a method for the quantitative estimation of sugar. His solution consists of a strongly alkaline solution of mercuric cyanide. The method has not, however, come into general use, at least not for biochemical work. For the solution is stated [Browne, 1912] to be reduced not only by sugars but also by creatinine, glycerol and a number of other organic substances other than sugars, and is therefore unsuitable for biochemical work either as a qualitative test or as a quantitative method. The same objection applies to the modifications of Knapp's solution by Sachsse and Bauer [quoted by Browne, 1912]. From the observations recorded in this paper it would appear that the non-specific nature of Knapp's solution is due mainly to its strong alkalinity. Indeed, if the reagent described in this paper is made very strongly alkaline by adding strong caustic soda, it is readily reduced even by glycerol. Observations are at present being carried out to determine whether the reaction described in this paper is suitable for the quantitative estimation of sugar in urine and other physiological fluids.

In conclusion the advantages of the test described in this paper in comparison to Fehling's test and Nylander's test as applied to urine may be stated. They are as follows:

1. The test is free from the fallacies inherent in Fehling's test as the result of the reducing action of uric acid and creatinine on Fehling's solution.

2. It is more easily carried out than Nylander's test, especially when small amounts of sugar are tested for.

3. With small quantities of sugar it is easier to judge roughly the amount of sugar present from the degree of turbidity obtained in this test than from the changes observed in Fehling's or Nylander's test. The test is in fact particularly suitable when the amounts of sugar present exceed the normal amount only slightly; that is to say in those conditions where Fehling's test gives ambiguous or doubtful results.

4. It is more sensitive than Fehling's or Nylander's test and allows a distinction to be made between the amount of sugar normally present in urine and that slightly exceeding the normal.

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XVI. ON A GREATLY IMPROVED HAEMIN TEST FOR BLOOD, WITH NOTES ON SOME RECENTLY PROPOSED METHODS.

By WILLIAM BEAM and GILBERT ARTHUR FREAK.

(*Received February 24th, 1915.*)

Since Teichmann in 1853 brought forward his test for blood by the production of haemin crystals, a host of modifications have been suggested by as many writers, and a fresh crop of the same appears each year. This in itself is sufficient indication that the method in its original and modified forms is far from satisfactory. Indeed, even an experienced and skilful worker may, apparently most unaccountably, fail to obtain a positive result, though working with fresh and unaltered blood specimens. With those encountered in ordinary laboratory practice, often minute in quantity and altered by age, the action of heat or sunlight, or the presence of rust or earth, or consisting of stains partly washed out by soap and water, the difficulties and uncertainty are increased to such an extent that in many laboratories the method has fallen more or less into disrepute. A number of sorting tests, presumptive only, have taken its place, the final decision resting upon the detection of corpuscles by the microscope, some form of spectroscopic examination—often exceedingly difficult with the limited amount of material at hand—or, in certain cases, the use of "precipitin" reagents.

None of the above-mentioned sorting tests, e.g. those employing guaiacum, benzidine, leuco-base of malachite green, phenolphthalin, etc., can satisfactorily replace the haemin test, since the latter alone affords absolute and unmistakable proof of the presence of blood.

In a hot dry climate like that of the Sudan, blood stains alter more rapidly than in more temperate zones, and the difficulties experienced with the haemin test are therefore usually greater. The need for some more reliable method than any of those yet advanced was actually felt, and in

the investigation which was consequently undertaken, the following considerations were kept in mind.

(*a*) The amount of blood present being usually small, it is essential that the whole of the portion taken be brought into solution. To insure this the acid must remain in contact with the suspected material for a long time—some hours at least.

(*b*) For the reason already stated above, the operation must be carried out so that no loss may occur.

(*c*) In many cases, especially when the blood is mixed with rust, earth, etc., it is highly desirable that some means be provided for filtering the liquid.

(*d*) If crystals of good size are to be obtained from so small a bulk of liquid—a few drops at most—special precautions should be taken to insure slow evaporation. Slow evaporation has often been recommended but the means prescribed for securing it have been totally inadequate, the operation being completed in, at most, a few minutes. Our endeavour has been to extend this to hours or even, if necessary, to days.

All the above essentials have been happily provided for in the following simple procedure the success of which, in all classes of cases, has exceeded our most sanguine anticipations.

A small quantity of the suspected material is placed at the bottom of a flat arsenic sublimation tube about 6 by 3 mm. and 35 mm. long. A thin round ignition tube will answer if no flat tube is procurable, but for examination under the microscope the flat form is obviously better. A few drops of acetic acid containing from 0·01 to 0·1 per cent. sodium chloride are added and a very fine cotton thread adjusted so that its upper end is near the top of the tube and the lower end reaches to the bottom of the liquid. The thread should be everywhere in contact with the tube, to which it adheres readily by being moistened with the liquid (Fig. 1). The adjustment is readily made by means of a glass rod one end of which is drawn out for the purpose. The tube is now placed in a rack, or supported by forcing its base into a small blob of plasticine, and allowed to remain until crystallisation has taken place.

Before placing the thread into position, the solution of the blood may be aided, if desired, by cautiously heating the tube over a small flame, loss of the liquid by explosive boiling being prevented by holding the finger tightly over the mouth of the tube.

While such heating appears rarely to be essential, the long contact with

the acid in the cold sufficing for solution in practically all cases, it is nevertheless advisable in that it gives at once an indication of the strength of blood solution obtained. For the best results this should be quite weak.

The clear liquid, filtered by its passage through and along the thread, is slowly drawn up, by capillary action, to the mouth of the tube. Under these conditions evaporation takes place so slowly that, even when the temperature of the atmosphere is above 38° C., it is complete (if the tube is held in an upright position) only after 12 to 24 hours or more. The rate

Fig. 1. Actual size.

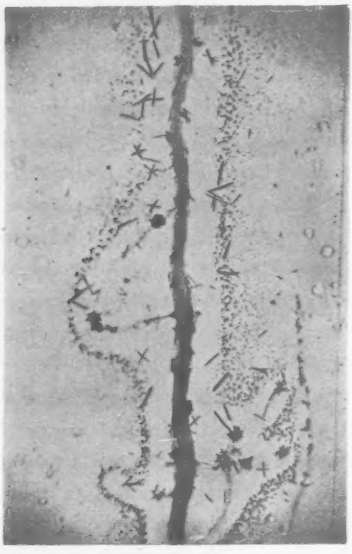

Fig. 2. ×25.

of evaporation is readily controlled, within wide limits, by altering the dimensions of the tube and the angle at which it is supported.

The crystals usually begin to appear, on or near the upper half of the thread, and of sufficient size to be distinguished with a power of 75 diameters, in about an hour; and they ultimately become so large that, in place of requiring a magnification of 250 to 300 diameters, as usually recommended, they may readily be seen with one of 25 diameters or even, in many cases, with a good hand lens (Fig. 2). The crystals are best viewed against a dull white background. A piece of white paper is suitable.

The extraordinarily large size of the crystals is due, in great part, to the slow evaporation of the liquid; but it is also a result of the removal, by the thread, of the interfering action of the blood albumin on crystallisation. Examination of the thread at the end of the experiment shows that the soluble, non-crystallisable, constituents of the blood are carried to its upper end, and that the maximum growth of haemin crystals is found lower down.

If the solution is sufficiently weak the first crystals form at the point of greatest concentration—the upper end of the thread; but as the albuminous matter accumulates these cease to grow and the largest and most numerous crystals are found, later, about midway down the tube.

For the best results the following precautions should be observed.

The tube should be thoroughly clean and dry. It is best cleaned by swabbing out successively with acetic acid, water, and alcoholic soda, followed by thorough rinsing with distilled water and drying on a hot plate.

The thread should be clean and as fine as can be obtained. A good plan is to take No. 80, "six cord sewing machine cotton" and to untwist it, using one of the "cords" for the test.

The solution of the blood in acetic acid should not be concentrated, but very weak. The liquid should only be faintly pink. This insures large crystals and clean fields.

If the method is to be applied experimentally to fresh blood, the latter must be completely dried before the addition of the acid, and especial care should be taken to work with very dilute solutions.

The test has been applied as described to:

(1) Human blood stains—fresh.

(2) Human blood mixed with earth—a forensic case.

(3) Human blood stains—10 years old.

(4) Sheep's blood stains—fresh.

(5) Sheep's blood stains—18 months old.

(6) Sheep's blood stains, heated to 110° C. for 1 hour.

(7) Sheep's blood stains on a rusty knife, two months old and also exposed to the air and sun for several days.

(8) Sheep's blood stains which had been, in great part, removed by washing with soap and water.

(9) Cow's blood stains—12 years old.

(10) Gamoose's blood stains—12 years old.

(11) Dog's blood stains—two months old.

Upwards of 300 trials have been made, using the method as described or slight modifications of it, and in no single case did the crystals fail to appear.

Many trials were also made with modifications employing other acids in place of acetic and other forms of halide. These experiments need not be recorded in detail, but the following are some of the conclusions arrived at.

Acetic acid without any saline addition will suffice for stains, fresh or old, which have not been treated with water, the salts present in the blood being sufficient for the test. Stains thoroughly washed with water will not respond unless some salt, chloride or bromide, is added.

As halides, the following were tried. To make the comparisons conclusive the blood stains were previously thoroughly extracted with water. Unwashed stains were also employed.

(*a*) Hydrochloric acid and hydrobromic acid.

(*b*) Sodium fluoride.

(*c*) Ammonium, sodium, potassium, calcium and magnesium chlorides.

(*d*) Ammonium, sodium and potassium bromides.

(*e*) Sodium and potassium iodides.

Hydrochloric and hydrobromic acids gave invariably negative results.

Sodium fluoride gave positive results in some cases, but inferior to those with chlorides or bromides.

All the chlorides and bromides tried gave good results. With magnesium chloride and calcium chloride (one tenth per cent. solutions) there is a marked tendency to the formation of a greater number of crystals, of smaller size but long and very slender.

The iodides, with washed blood stains, usually gave negative results. In some cases a very few small crystals were formed but these appeared to be due to the incomplete removal of chloride from the blood, or its presence as impurity in the reagent. With unwashed stains iodides gave results inferior to those with chlorides or bromides. Similarly a reagent composed of a mixture of the three halides gave results in some cases negative and, in all, inferior to those with chloride or bromide alone. From these results the conclusion appears obvious that the presence of iodide in the reagent is distinctly contra-indicated.

As substitutes for acetic acid, formic and propionic acids were tried. Lactic acid, mentioned by Teichmann and recommended recently by Symons (*vide infra*), is obviously not suitable for this form of the test as it is not sufficiently volatile.

The results with formic acid, alone and with the addition of the various halides mentioned above, were invariably negative.

Propionic acid gave results, in some cases negative, and in all cases inferior to those with acetic acid.

In short, of the solvents experimented with, acetic acid was found to be by far the best and is in fact, for this form of the Teichmann test, the only reliable one. Further, no appreciable advantage was found to result from the substitution of other forms of halide for sodium chloride.

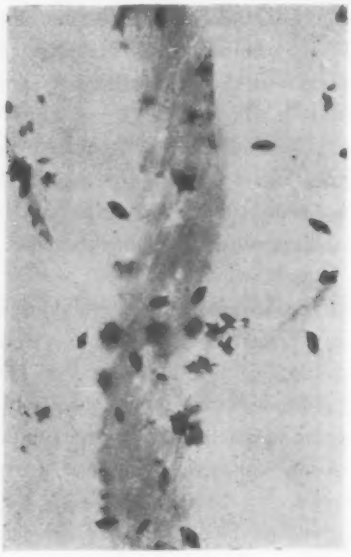

Fig. 3. ×50.

It was noted that in the case of very old blood specimens there is a marked tendency to the formation of short, broad haemin crystals and also of hemp seed forms (Fig. 3).

Notes on some recently Proposed Methods.

Nippe [1912] recommends the following reagent as affording a "ready and certain" means for the identification of blood stains: potassium bromide 0·1 g.; potassium iodide 0·1 g.; potassium chloride 0·1 g.; glacial acetic acid 100 g. A drop of the solution of the stain in the reagent, on a

microscope slide, is covered with a cover glass and warmed until bubbles just form. "As it cools the growth of the haemin crystals can be watched under the microscope."

It is not easy to see any scientific basis for this modification of the reagent. In our hands it has been found to offer no advantages whatever and the method as a whole suffers from all the disabilities of the original procedure. In the light of the experiments already detailed it would seem likely that any positive indications were obtained, not as a result of the presence of iodide, but in spite of it.

W. F. Whitney [1912] published a note on a method of performing the haemin test which appeared to have advantages. It was noted that formic acid of sp. gr. 1·2 when placed on a glass slide and allowed to evaporate, disappeared gradually from the periphery to the centre. A drop of acetic acid, on the other hand, did not hold together but spread over the slide and evaporated instantly. Formic acid appeared therefore to be a better solvent for the blood, as it permitted of concentration of the solution and the deposition of the crystals in a much more confined area. The method recommended was as follows. A minute fragment of the suspected substance —a scraping from a stain or a bit of stained fibre—is placed on a slide and a small drop of formic acid applied with a glass rod. Over the drop is inverted a small watch glass which should not touch it nor project beyond the edge of the slide. Gentle heat is applied beneath the drop and the moment condensation is seen inside the watch glass, the heat is discontinued, the watch glass removed, and the remainder of the liquid allowed to evaporate in the air. When dry the spot is examined under the microscope. The crystals are said to be small, requiring a high power objective to see them well, but so numerous that they cannot be overlooked.

The remarkable statement is added that "the fact they are so small shows that only a minute quantity of blood is necessary for their formation."

We have made many trials of the above method with most disappointing results. Haemin crystals were obtained in one case using quite fresh blood of which a larger quantity was taken than is usually available for test. Aside from this all trials were fruitless, although the field was searched most carefully under a 1/7 inch objective. Even when precautions were taken to insure much slower evaporation than is possible with the method as detailed, the issue was still wholly unsatisfactory.

These results bear out those obtained by the method detailed in the body of the paper which indicate that formic acid cannot safely be substituted

for acetic acid for the haemin test. This point was very thoroughly investigated by the use not only of formic acid alone but of formic acid containing hydrobromic acid, sodium fluoride, sodium chloride, potassium bromide and potassium iodide. In no case were haemin crystals formed either with fresh or with old blood stains.

An improvement on the procedure as recommended by Whitney was effected by substituting for formic acid a mixture of equal parts of formic acid and acetic acid, the test being applied as described except that the slide is best slightly heated before the drop of liquid is placed upon it. The results were, however, at best, unsatisfactory and the method failed entirely in the case of old stains or those which had been partially washed out.

A far better method than either of the above, but taking advantage of the same principle of prevention of spreading of the liquid, is the following. The solution of the suspected material is effected in a very small test tube, such as an ignition tube, the reagent being a few drops of acetic acid containing 0·01 per cent. of sodium chloride. The tube should be heated cautiously over a small flame, or for ten or fifteen minutes in a steam bath or oven, in order to insure complete solution. The liquid is then transferred by means of a small pipette to a glass slide coated with a very thin layer of paraffin wax, and set aside in a place protected from dust, for evaporation. The amount of wax on the slide should be so small as to be only just visible. The slide may be prepared by melting on it a small quantity of wax, the excess of which is then removed by means of a dry cloth.

The rate of evaporation of the liquid will depend upon the amount of paraffin wax on the slide. It has been found that the wax dissolves in the acetic acid, and after the first rapid concentration has taken place the liquid becomes coated by a continuous film of wax which checks evaporation to such an extent that it is in some cases not complete even after two or three days. Under these conditions the crystals formed are usually few in number but very large. With less paraffin wax present, so that evaporation is complete in about an hour, crystals are formed of sufficient size to enable them to be distinguished by a magnifying power of about 50 diameters.

The method has been found to be applicable even to very old blood stains, which points clearly to the fact that the difficulties experienced with the ordinary haemin test, when so applied, were not due, as has been frequently claimed, to decreased solubility of the blood, but rather to too rapid evaporation of the solvent.

The method is, as stated, a marked improvement on the ordinary one; but it is not intended to recommend it in preference to that described above, which is far superior to it on the score both of reliability and delicacy.

C. T. Symons [1913] recommends the following modification of Teichmann's test as especially suited to old stains which have been exposed for a long time to air and sun in the tropics: "Solid sodium iodide, freshly prepared, is dissolved in lactic acid (sp. gr. 1·2) to about one per cent. The solution becomes brown on keeping but this change does not appear to impair its action, at any rate for some months. The fragment of stain is covered with the solution, the cover glass is placed in position and the whole slowly warmed to such a temperature that the solution is just about to boil under the cover glass. In most cases five minutes' treatment is sufficient to produce crystals. If they have not then appeared the heating must be continued; more solution must be allowed to run under the cover glass if necessary, but this is not often the case as the lactic acid does not evaporate as rapidly as acetic acid. The resulting crystals differ very much in size in different cases, being usually rather small, but dark and perfectly characteristic."

In our hands this method gave, at first, very unsatisfactory results. It failed entirely in the case of old stains and even with quite fresh specimens positive indications were by no means the rule. Later, using another sample of lactic acid, much better results were obtained. It was found however that success was to a great extent dependent upon the control of the heating. A small water oven containing a saturated salt solution (boiling at about 107° C.) is probably the safest means, but the temperature of an ordinary water oven is usually sufficient. The best results were obtained not, as recommended, by long heating but by heating for about five minutes only. In some cases, when heating was prolonged, what appeared to be crystals too small for positive identification were formed, but disappeared entirely on further heating. Success in ordinary cases appears to depend upon the entanglement of a supersaturated liquid in the meshes of the fabric. In other words if the blood material is allowed to become diffused throughout the entire mass of the liquid under the cover glass, no crystals may be formed.

"Freshly prepared" sodium iodide is prescribed for the reagent. In our experience this has not been found to be necessary, the salt which had been kept a long time answering equally well.

SUMMARY.

(*a*) Of the reagents which have been recommended for use in Teichmann's test for blood, acetic acid is by far the best and is the only one on which entire reliance may be placed if the method by evaporation is employed.

(*b*) Acetic acid, without salt of any kind, suffices for the test with blood stains, fresh or old, provided the stains have not been extracted with water. As a precautionary measure it is best however to use a reagent containing a minute proportion—about 0·01 per cent.—of sodium chloride.

(*c*) No advantage results from the substitution of other salts for sodium chloride in the acetic acid reagent.

(*d*) Iodides may not be used to substitute chlorides or bromides in the above. This does not apply to the reagent made with lactic acid.

(*e*) Where positive results have been obtained from the use of an acetic acid reagent containing iodide, the effect is apparently due to the chloride naturally present in the blood, or as impurity in the reagents.

(*f*) The difficulties experienced with Teichmann's test when applied to stains, both fresh and old, are due chiefly to the too rapid evaporation of the solvent and, to a less extent, to interference of the albuminous matter of the blood with the crystallisation. Evaporation should be extremely slow and when carried out in the manner detailed, which also eliminates the harmful effect of albumin, crystals are obtained with the greatest certainty and of remarkably large size, even though only a minute amount of blood be present.

(*g*) The test as described was found to be equally applicable to blood stains fresh or old (12 years), stains partially removed by soap and water, or heated to 110° C., or mixed with earth, or to old stains on rusty iron which had been exposed to strong sunlight and atmospheric conditions for several days.

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XVII. A STUDY OF THE METABOLISM IN EXPERIMENTAL DIABETES.

By VICTOR HENRY KINGSLEY MOORHOUSE,
SIDNEY WENTWORTH PATTERSON,
AND MARJORY STEPHENSON,

Beit Memorial Research Fellows.

From the Institute of Physiology, University College, London.

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I. Introduction.

The diabetes produced in dogs by the removal of the pancreas has been the theme of many investigations which have shown how similar the condition is to a severe type of human diabetes and have given hope of an explanation upon similar grounds. The questions which investigation has left debatable concern the relative importance of two factors which might be primary causes. These factors are:

1. An over-production of sugar by the organism.
2. A loss of the power to use sugar by the tissues.

According to the over-production theory the removal of the pancreas does away with a normal control exerted upon protein and fat metabolism and results in a flooding of the organism with sugar formed from these substances so that the limit of sugar usage is exceeded and a portion of the sugar wasted. The non-utilisation theory presupposes that the internal secretion of the pancreas is indispensable to the tissues in the combustion of carbohydrate. The over-production theory might well be accepted as a working hypothesis but for certain objections. These are the low level of the respiratory quotient, which points to non-utilisation of carbohydrate although the blood is hyperglycaemic, and the steadily increasing D : N ratio in the course of the diabetes when no carbohydrate is given. It is also very plain that the over-production theory would be difficult to prove until the question of the utilisation power had been settled.

The problem of utilisation has been attacked in three ways. The first method was to administer sugar and see how much of the dose could be recovered from the urine. Minkowski based his conclusion as to the non-utilisation of glucose upon results from this method, and his observations have been confirmed many times. The second method consists in observations upon the blood-sugar content. Starling and co-workers estimated the amount of sugar used by the heart by the fall in blood sugar level during an experiment with the heart-lung preparation. In the first series of experiments of this type Knowlton and Starling [1912] found evidence of a loss of the power of sugar utilisation by diabetic hearts. Later work has shown that sugar disappears from the blood perfusing diabetic hearts and has pointed out the possible sources of error in experiments of this type [Patterson and Starling, 1913; Macleod and Pearce, 1913]. Whether conclusions as to sugar usage can be safely made from the disappearance of reducing substance from the blood in such experiments is still a matter for consideration. The third method of investigating sugar utilisation in diabetes is by observing the respiratory exchanges of the intact animal or isolated tissue. The determination of the respiratory quotient gives a definite measure of the extent to which carbohydrate is being made use of by the organism. Evans and Starling [1914] have studied the respiratory exchanges of the heart-lung preparation in a series of normal and diabetic dogs. They find that the respiratory quotient of the diabetic preparation is lower than the normal, taking averages of the large series of observations. They conclude that the diabetic heart shows little evidence of sugar utilisation because of the low level of the respiratory quotient in spite of the fact that the blood is hyperglycaemic. Porges and Salomon [1910] maintain that the carbohydrate utilisation is unimpaired, since they observed high respiratory quotients in diabetic dogs whose abdominal organs were excluded from the circulation. Murlin, Edelmann and Kramer [1913] point out, however, that a high respiratory quotient in such a condition is of no significance as regards sugar usage. It is evident, as these authors state, that the abrupt change in circulatory conditions, coupled with respiratory and blood reaction variations incident upon the clamping of the abdominal vessels, must be followed by an abnormal respiratory exchange. In the extensive observations of Benedict and Joslin [1910, 1912] upon human diabetes the severity of the disease has been found to increase with the lowering of the respiratory quotient. The respiratory exchange in diabetes indicates a loss or an impairment of the power of sugar utilisation, at least in the light of our present knowledge.

In view of the unsettled state in which the question of sugar usage in diabetes still remains it seemed desirable to undertake an investigation of the metabolism in dogs before and after depancreatisation. Observations were made upon the respiratory exchange of these animals and detailed analyses of the urine and faeces carried out. The main objective has been the question of sugar utilisation. The results are reported in the following paper.

In this report we wish to acknowledge gratefully the considerable assistance we have received from the members of the staff and other workers at the Institute of Physiology. Dr Plimmer and Mr Reeve have done the urine analyses throughout the research. Dr Cruickshank carried out the tissue and faeces analyses. We take pleasure in thanking these and all the workers at the Institute for help in every stage of the research. We are greatly indebted to Professor Starling, at whose suggestion the work was undertaken, for performing the necessary operations and for his help and advice throughout the research[1].

II. Methods.

Animals. Bitches were used in all the experiments. Care was taken in the selection of young and normal animals and in training them to lie quiet in the apparatus. While under observation the dogs were kept in metabolism cages in a well-lighted, heated and ventilated room.

Feeding. The animals were usually fed once a day at about 5 p.m., no food being left in the metabolism cage over night. The food given was carefully weighed and precautions were taken to prevent wasting of the weighed amount.

Diets and Food Analyses. The diet given to normal dogs varied in character for purposes of experiment. Full notes of the diet will be found in the protocols. The standard diet given to diabetic animals was chopped horse meat and ox pancreas, in the proportion of four to one. Samples of the food were analysed from time to time and estimations of total nitrogen, carbohydrate, phosphate and fat were made where necessary. The following table gives these analyses and the corresponding abbreviations will be used in the protocols when referring to these foods.

[1] Dr Plimmer wishes to thank the Government Grant Committee of the Royal Society for the grant defraying the expenses of the chemical work in connection with this research.

The expenses in connection with the analyses of fats were partially covered by a grant from the Royal Society to Dr Cruickshank.

TABLE I.

	Abbreviation	N %	Carbohydrate %	P_2O_5 %
Horse meat	M.	4·0 to 4·4	—	0·5 to 0·66
Ox pancreas	P.	2·89	—	1·16 to 1·30
Caseinogen	C.	11·0	—	—
Gelatin	G.	14·0	—	—
Puppy biscuit	P.b.	3·2	60·0	—
Palmine	Pal.	—	—	—
Cod liver oil	C.L.O.	—	—	—

Sugar administration. The sugars administered were glucose and fructose The dose given was 20 g. by the mouth, as the dogs were found to take this amount dissolved in water readily, and during succeeding respiratory observations would remain quiet in the apparatus. Rectal enemata of sugar solutions were also given, but owing to difficulties in controlling the amount given, liability of the enemata being expelled and varying rate of absorption, the results cannot be regarded as regular enough for comparison. With intravenous injections difficulties arose from the subsequent uneasiness of the animals in the apparatus. The effects of intravenous sugar on the respiratory exchange of diabetic animals will be studied in a future research. The standard dose of glucose or fructose in these experiments was 20 g. given per os.

Operations. The operations were all performed by Professor Starling. The dogs were given a small dose of morphine about two hours before the operation and full anaesthesia was produced by ether-chloroform mixture. Due aseptic precautions were taken and no serious wound infections were observed. All the animals recovered quickly from the operation and showed no uneasiness on the following day.

The method of Hédon was employed in the total extirpations of the pancreas and six dogs were operated upon in this manner. They were dogs I, II, III, IV, V, VI.

Two dogs, VII and VIII, were partially depancreatised and as the procedure was different the operations will be described separately.

Dog VII. All pancreatic tissue was removed from the abdominal cavity, leaving a small portion of the tail, about one square centimetre, free with its blood supply intact in a pedicle of mesentery. This portion was implanted under the skin of the abdominal wall. The graft was functional, because there was a discharge of clear juice for a short period after the operation. This juice digested fibrin slowly. The appearance of sections of the graft is

noted later. In histological structure the tissue showed little evidence of degeneration a month after the operation.

Dog VIII. This animal had two operations performed on it.

Operation 1. All the pancreatic tissue was removed with the exception of a mass about the size of a pea, which was left close to the main pancreatic duct. The duct was ligated on the peripheral side of the mass. This small portion was evidently functional as the dog, after having a slight glycosuria for a week, became apparently normal and only showed a slightly reduced sugar tolerance for glucose and fructose.

Operation 2. The remainder of the pancreas was removed and the abdominal wound again closed. The dog immediately developed a marked glycosuria with a D : N ratio of 3·2, but at the end of two weeks seemed in far better condition than is usually the case with totally depancreatised animals.

Collection of urine and faeces. The dogs were kept in metabolism cages while under observation. They were catheterised at the end of twenty-four hour periods at 10 a.m. After catheterisation the animals were allowed to run round a room for a short period and almost always faeces were passed at this time. Rarely the faeces were passed in the metabolism cages. The cages were thoroughly cleaned every day. Before the animals could be catheterised easily it was necessary to perform an operation under anaesthesia in which the perineum was split and the vulvar opening thus widened so that a glass catheter could be readily passed. In some instances the animal was catheterised at intervals of about four hours during the day, so as to ascertain the nitrogen excretion during respiratory observations. On these occasions the figures from the analyses are added up to obtain the whole 24 hour results. While the animals were in the metabolism cages the urine was received into bottles through a layer of filter paper, and some crystals of thymol were placed in the bottles to inhibit bacterial growth.

Weight of animals. The animals were weighed carefully before observations in the respiration apparatus. The weighing was generally carried out in the morning after catheterisation and when faeces had been passed and before feeding.

Urine analyses.

Total nitrogen; Kjeldahl's method.

Ammonia; Folin's method.

Urea; Soya bean urease method, as described by Plimmer and Skelton [1914, 1].

Allantoin was estimated by difference between the figures obtained for urea by Folin's method and by the urease method [Plimmer and Skelton, 1914, 1]. For the estimation of allantoin in diabetic urine the method has been modified by Plimmer and Skelton [1914, 2].

Creatinine; Folin's colorimetric method.

Uric acid; Folin's colorimetric method.

Amino nitrogen; estimation by the difference between the formaldehyde titration and the figure for ammonia by Folin's method.

P_2O_5; Neumann's method as modified by Bayliss and Plimmer.

Acetone and acetoacetic acid; two distillations, the first from acid and the second from alkaline solution, were carried out. The amount of total acetone was estimated by Messinger's iodometric titration. Due precautions were taken to have the tube under the surface of the fluid in the receivers.

Hydroxybutyric acid; estimation by the extraction method of Hurtley. The estimation was discontinued because of the very small quantity present in the dog's urine.

Sugar; the figures obtained by Benedict's method are those given in the tables. The estimations were controlled by the use of Bertrand's method, polarimetric readings, and fermentation in some cases.

Faeces.

Fat; estimation of fat in the dried sample by the Kumagawa-Suto method.

Tissues.

Glycogen; estimation in heart and liver tissue was carried out as described by Cruickshank [1913].

Fat; the method of Kumagawa using Mottram's modification for the removal of unsaponifiable and resinous substances.

Respiration apparatus.

The small respiratory calorimeter devised by Benedict [1912] for animal experimentation was used. The reader is referred to Benedict's articles for a complete description of the apparatus. The construction of the apparatus is essentially the same as that described by Benedict, the only modification being an arrangement for regulating the temperature of the chamber in which the animal is placed. Two incandescent lamps were fixed inside the chamber and the amount of heat given off by them was regulated by a lamp and coil resistance interposed in the circuit outside the box. A thermometer

reading to tenths of a degree centigrade was placed inside the chamber and a temperature of about 24° C. was maintained during the observations.

Testing the apparatus. The apparatus was tested in the various ways mentioned by Benedict. The parts were first connected to a water manometer and subjected to a pressure of about 100 cm. of water. When the parts were made air-tight separately the whole apparatus was set up and blank determinations made. The weight of the carbon dioxide absorption sets was checked during these blank periods, so as to ensure full absorption of water vapour in the large sulphuric acid bottles. Also the temperature of the box was maintained constant and the level of the spirometer observed for long periods to make sure that the apparatus was air-tight. Finally, an alcohol lamp was burnt inside the chamber and carbon dioxide and oxygen measurements carried out. This test was frequently done in the course of the research and is a very valuable indication of the reliability of results.

Carbon dioxide measurements. The carbon dioxide was estimated by the gain in weight of the soda lime bottle and Williams flask together. The weighings were carried out to 0·01 g. on a large balance capable of weighing ten kilograms. The absorption set was carefully cleaned especially at the coupling joints before every weighing. The blank of determinations showed an error of ± 0·02 to 0·05 g. The absorption set weighed four to five kilos.

The soda lime was made according to the directions given by Benedict and the absorption set was frequently replenished with fresh material. The limit of absorption of the set is about 75 g., but the bottles were replenished before they had increased in weight 50 g.

Oxygen measurements. The oxygen used was that prepared by the electrolytic process and was 99 % pure. It was sent into the apparatus through a gasometer reading to 10 cc., which had been tested for its mechanical error at the ordinary room temperature and for various rates of flow of the gas. This error was:

at rates of 50 to 60 cc. per min.—reading × 0·992.
at rates of 70 to 80 cc. per min.—reading × 0·993,

In many experiments the oxygen was supplied from a small cylinder and the readings of the gasometer were checked by the loss of weight of the cylinder. When the results were satisfactorily checked the readings of the gasometer, corrected for mechanical error, temperature and pressure, were taken, and a large oxygen cylinder could be used.

The spirometer pointer was levelled at the beginning of periods with a fixed pointer and at the end of periods brought to lie along this level again.

One millimetre rise or fall of the spirometer represents 22 cc. It was found that one-tenth of a degree rise or fall of temperature in the chamber caused a movement of the spirometer corresponding to 30 cc. Care was therefore taken to maintain a constant temperature during periods of observation.

General technique of observations. The periods were usually from 40 to 60 minutes in duration. If the animal became restless towards the end of a period the observation was lengthened in order to avoid as much as possible errors from increases of lung ventilation and from changes of temperature in the chamber. The normal animals show much more restlessness than the diabetics, so that many normal observations were necessary to accustom the animals to the apparatus and thus obtain observations when the animals were quite quiet. The arrangement for recording movement described by Benedict was used and is useful as a rough means of comparing observations.

III. Results.

1. *Respiratory Quotient of Fasting Animals.*

Since the respiratory quotient is a varying ratio depending upon the extent to which protein, carbohydrate and fat are being oxidised in the organism, or being laid down as stores in the depôts of the body, it is very necessary to determine this ratio when the conditions of absorption and utilisation are as uniform as possible. In the fasting state when these conditions are most regular the respiratory quotient can be used as a basis of comparison if certain factors are controlled. The factors which can be controlled are the character of the diet and the time of the observation after a meal. Other factors which must be taken into account are the amounts of carbohydrate and fat stored in the body and available for usage. The 'fasting' respiratory quotients here referred to are observations made at least 18 hours after a meal and under conditions as nearly comparable as possible. In normal animals when meat is fed, the level of the 'fasting' quotient is found to be 0·72 to 0·77 with the usual figure of 0·75. When increasing amounts of meat are fed the quotient is somewhat higher. When large amounts of fat are given the quotient observed is 0·72 to 0·74 in the fasting state, so that whether protein or fat form the main part of the diet of dogs the character of the fasting metabolism appears to be much the same. When starchy foods are fed, the level of the 'fasting' quotient rises to 0·76 to 0·80. In the latter case evidently more carbohydrate material is available for usage or has been stored as glycogen and is taking a uniform part in the

metabolism, or perhaps some formation of fat from available carbohydrate is taking place. With the 'fasting' respiratory quotients of diabetic animals two points will be observed in the curves and protocols:

(i) the average value is about that observed in the normal combustion of fat, namely, 0·705;

(ii) the respiratory quotient varies within smaller limits than that of normal animals, viz. 0·68 to 0·74.

The lowest fasting quotient of dogs upon a diet rich in fats was 0·690, while the lowest diabetic figure was 0·670. Since the diet of the diabetic

Dog I.

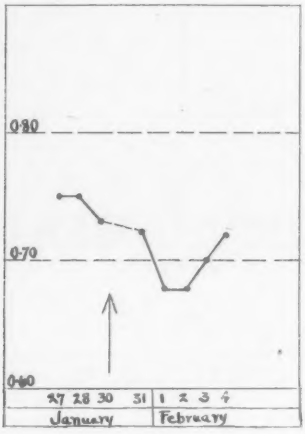


Fig. 1 *a*.

dogs was protein we must compare the 'fasting' quotients with the normal upon protein diet, which shows the diabetic quotient to be at a slightly lower level.

In Fig. 1 (*a* and *b*) the lower level of the 'fasting' quotient of totally depancreatised animals is clearly illustrated, and in most cases this level was lowest soon after the operation and tended to rise later. This rise may be only an apparent one, however, and not due to an increase in the ability to utilise carbohydrate but to a relative decrease in the amount of oxygen taken up. Later on in the course of the severe diabetes produced in totally depancreatised animals, the circulatory weakness and changes in the reaction of the blood evidently combine in causing a diminution in the oxygen intake and thus an apparent rise in the respiratory quotient.

With the partially depancreatised dogs the course of the fasting quotient varies. Fig. 2 from Dog VII shows that the quotient fell gradually to a low

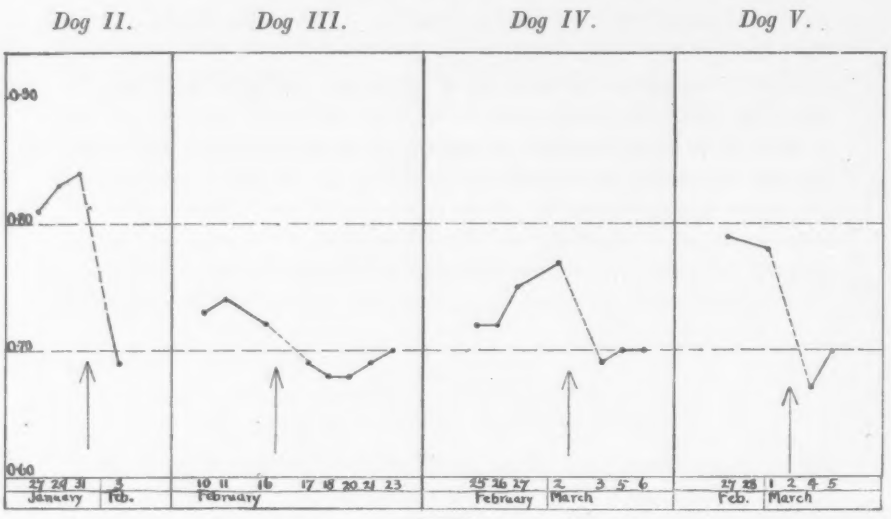


Fig. 1 *b*.

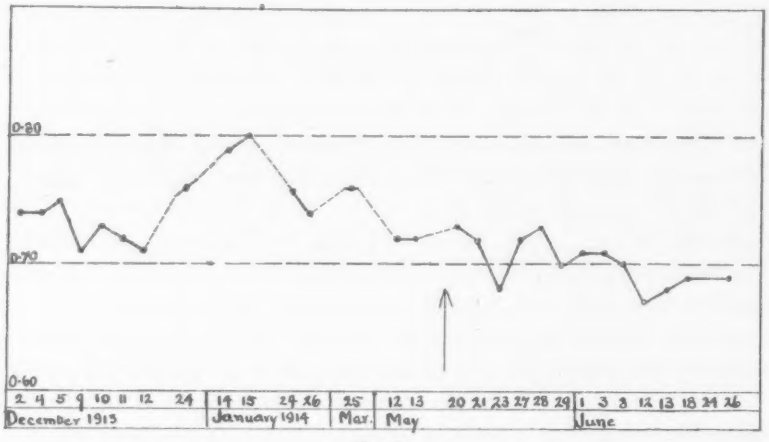


Fig. 2.

level as time went on. The course of the diabetes in this dog was very like that produced by total depancreatisation, except that the animal survived

longer. With Dog VIII partial depancreatisation produced a very transient and slight glycosuria. Fig. 3 shows that the respiratory quotient reached very low levels soon after the operation and then rose to normal and high levels. After total depancreatisation the course of the quotient was generally higher in Dog VIII than that observed in any of the other diabetic animals, and the general condition of the animal was much better than that of any of the others.

In Figs. 1, 2 and 3, the arrows show when the operations of depancreatisation were performed.

Dog VIII.

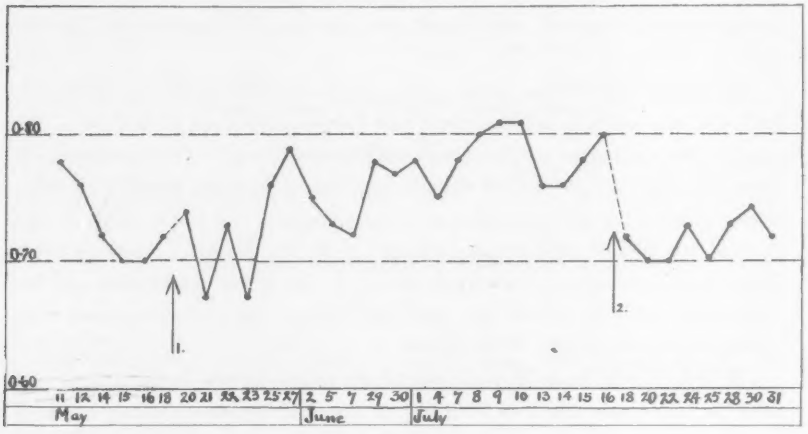


Fig. 3.

2. *Total Metabolism as judged by Oxygen Consumption.*

The figures for the oxygen consumption in the following table (II) are given in cc. per kilo. per hour. They are in every case from observations upon the animals at least 18 hours after feeding and necessarily when the animals showed the greatest degree of rest, and when the figures showed a uniformity in successive periods. Thus the first observation after the animal has been placed in the respiratory chamber has not been used on account of the usual initial restlessness, and the figures for oxygen consumption are an average of successive quiet and uniform periods. The results are therefore comparable with a fair degree of accuracy. Before remarking upon these results it is necessary to mention that there are certain objections to a comparison between

the total metabolism of normal and diabetic animals based upon the oxygen consumption in cc. per kilo. of animal per hour.

(1) The surface extent of the animal remains approximately constant while the weight of the animal decreases very rapidly when diabetes ensues. There is therefore a relatively greater area where heat loss may occur and one might expect a compensation for this in an increase of metabolism per unit of weight.

(2) The loss in weight in diabetes chiefly concerns water and fat which in the normal condition are in themselves not active consumers of oxygen, while organs and tissues which are constantly and actively engaged in metabolism preserve their normal rate of oxygen consumption. If this occurred, an apparent increase of total metabolism per unit of weight would be the result.

Another consideration in the comparison of total oxygen figures is the fact that the animals become more and more accustomed to and therefore quiet in the apparatus as the observations are continued. This consideration does not apply in the case of the partially depancreatised dogs which were under observation for some time as normal animals. It would apply in the case of the totally depancreatised dogs. Also the depressed state of these diabetic animals must be taken into account. As far as observation and the kymograph records indicate the muscular activity, the diabetic animals were invariably quieter than the normal.

It will be seen from Table II that there is an increase in the oxygen per kilo. per hour amounting to 10–15 % in the diabetic state, taking averages of all normal and diabetic observations. It will be noted that the figures show a steady increase from the time of operation up to the termination. In the last stages it has been remarked that the oxygen intake may decrease markedly owing to circulatory disturbances. The increase in the total metabolism becomes more striking when we remember that the diabetic animals are always quieter than the normal. This increase in the total metabolism we think ought to be regarded as a real increase, not an apparent one, depending upon the two considerations mentioned above. A comparison of the weights and oxygen consumption of Dog VIII in Table II shows that a decrease of weight does not necessarily cause an apparent increase of the total metabolism. This table is divided into six sections so that the figures obtained after both operations and under varying conditions may be compared. It will be noted that a steady decrease in weight occurs in sections II and III without a parallel increase in the oxygen consumption.

This indicates that the part played by a decrease in the more inert elements of the body or a decrease in weight with the same surface area, in causing an apparent increase in oxygen figures per unit of weight, is at any rate a minor factor. The complications which septic infection of the animals would

TABLE II.

Weight (kilos.) and O_2 consumption (cc. per kilo. per hour).

Dog IV.

Normal				Diabetic			
Date	Weight	O_2		Date	Weight	O_2	
1913							
Feb. 27	8·000	541		Mar. 5	7·600	615	
Mar. 2	8·000	517		„ 6	7·300	591	
		529	Average			603	Average

Excess diabetic over normal O_2 consumption = 14 %.

Dog VI.

July 21	7·100	563		July 30	5·900	615	
„ 23	7·000	512					
		537	Average			615	Average

Excess diabetic over normal O_2 consumption = 14 %.

Dog VIII.

May 15	8·250	512	I. Normal	May 20	7·750	525	II. Partially depanc.,
„ 18	8·000	516		„ 21	7·550	518	slight glycosuria
				„ 23	7·400	532	
				„ 25	7·200	515	
				„ 27	7·000	438	
		514	Average			505	Average
June 2	6·750	490	III. No glycosuria	July 7	6·650	503	IV. On thyroid, slight
„ 5	6·750	431		„ 9	6·500	524	glycosuria
„ 9	6·750	458		„ 10	6·500	575	
„ 29	6·500	467		„ 13	6·350	535	
„ 30	6·500	466					
July 1	6·500	467					
„ 4	6·600	466					
		463	Average			534	Average
July 14	6·400	473	V. No glycosuria	July 18	6·250	493	VI. Totally depanc.
„ 15	6·450	450		„ 20	6·000	524	D : N = 3·1
„ 16	6·500	431		„ 22	5·800	548	
				„ 24	5·650	553	
				„ 25	5·600	560	Excess period VI over
				„ 28	5·350	552	period I = 8 %
				„ 30	5·200	590	
				„ 31	5·200	623	Period VI over V = 23 %
		451	Average			555	Average

TABLE II—*Continued*

Dog VII.

1913							
Dec.	5	10·050	543	May	20	8·650	535 Partially depanc. May
„	24	9·600	451	„	21	8·500	532 18th
1914							
Jan.	14	10·000	432	„	23	8·100	531
„	24	9·500	545	„	27	7·100	526
„	26	9·500	520	„	28	7·100	495
Mar.	25	8·700	451	„	29	7·050	524
May	12	9·150	488	June	1	6·900	524
„	13	9·150	479	„	3	6·750	501
				„	8	6·500	525
				„	12	6·200	576
				„	13	6·000	569
				„	24	5·200	528
				„	26	5·000	495
			488 Average				532 Average

Excess diabetic over normal O_2 consumption = 9 %.

Dog I.

1913							
Jan.	27	7·000	563	Jan.	31	6·400	628
„	28	7·000	579	Feb.	1	6·350	670
				„	2	6·100	688
				,	3	5·800	637
				,	4	5·600	664
			571 Average				657 Average

Excess diabetic over normal O_2 consumption = 15 %.

Dog III.

Feb.	10	7·600	518	Feb.	17	7·200	575
„	16	7·500	486	„	21	6·750	595
				„	23	6·500	594
			502 Average				588 Average

Excess diabetic over normal O_2 consumption = 16 %.

introduce in comparison of oxygen figures are absent, as the post-mortem examinations revealed no evidence of a generalised septicaemia.

3. *Percentage energy distribution.*

(*a*) *Protein.* From the data obtained in the measurement of the respiratory exchange and estimation of the respiratory quotient it becomes possible to calculate the percentage part of the total metabolism taken by protein, carbohydrate and fat. This calculation is made by subtracting from the total figures the amounts of carbon dioxide and oxygen corresponding to the protein metabolised and estimating the respiratory ratio of the remainder,

which indicates quantitatively the extent to which carbohydrate and fat are being used. An example may be given:

Dog VII. Dec. 3, 1913.

	CO_2	O_2	R.Q.
Fasting 23 hrs. Starch diet	4160	5418 ⟶	0·760
Nitrogen, g. per hour 0·110	537	664	
	3623	4754 ⟶	0·762

In this example we find that the protein portion amounts to 12 % of the total and the quotient of the remainder shows that the carbohydrate percentage is 17·0 and the fat 71·0 %. The figures for one hour are used. The nitrogen excretion is calculated in this example from the 24 hours analysis and the figures for CO_2 and O_2 corresponding to protein are taken from Schumburg and Zuntz's table quoted by von Noorden [1907]. It is evident that, in order to obtain a correct value for the protein percentage, the nitrogen excretion should be estimated during the respiratory observation. An estimate based upon the 24 hours nitrogen in the above example is not so liable to error as it would be if a protein rich diet were being fed. In the latter case such a nitrogen figure would give too high a percentage for protein during a fasting period and too low in observations after feeding. In the normal dogs 15 to 20 % was found to be the usual protein percentage of the fasting metabolism when meat was fed, and where direct determinations of

TABLE III. *Percentage energy distribution. Fasting observations. Normal.*

No. of animal	Date	Hours after feeding	Diet	N	Carbohydrate	Fat	% energy distribution: Protein	Carbohydrate	Fat	
	1914									
III	Feb. 10	18	P.b.	—	—	—	15·0	6·0	79·0	Protein p.c. assumed
	„ 16	17	Meat	4·0	—	—	22·0	0·0	78·0	
VI	July 23	23	Meat	8·0	—	—	15·0	12·0	73·0	„
	1913									
VII	Dec. 3	23	P.b.	4·5	120·0	—	12·0	17·0	71·0	
	„ 4	18	P.b.	1·6	60·0	—	12·0	8·0	80·0	
	„ 5	19	P.b.	3·3	135·0	—	13·0	13·0	74·0	
	„ 24	18	Mixed	11·0	70·0	—	15·0	14·0	70·0	„
	1914									
	Jan. 15	18	Mixed	4·7	60·0	—	15·0	30·0	55·0	„
	Mar. 25	24	C. and Pal.	6·6	—	40·0	15·0	15·0	70·0	„
	May 9	17	Meat	10·0	—	—	15·0	10·0	75·0	„
	„ 14	22	Meat	10·0	—	—	15·0	0·0	85·0	„
VIII	„ 18	24	Meat	8·0	—	—	15·0	3·0	82·0	„

nitrogen excretion have not been made, this figure (15 %) has been assumed. In the tables it is indicated where the protein percentage has been directly calculated and where it has been assumed.

Table III contains a summary of these calculations from the respiratory and nitrogen figures of normal fasting animals. In Table IV will be found the observations made after feeding protein and fat. The results obtained after administration of sugars will be found in the section dealing with carbohydrate.

TABLE IV. *Percentage energy distribution in digesting animals. Normal.*

No. of animal	Date	Hours after feeding	Diet	N	Carbohydrate	Fat	R. Q.	% energy dist. to Protein	Carbohydrate	Fat
	1914									
III	Feb. 12	4 to 6	Meat	4·0	—	—	0·806	44·0	19·0	37·0
	„ 13	5 to 7	Meat	4·0	—	—	0·798	41·0	18·0	41·0
	1913									
VII	Dec. 13	2–3	Meat	8·0	—	—	0·818	79·0	10·0	11·0
	„ 15	2–3	Meat	10·0	—	—	0·802	90·0	1·3	8·7
	„ 15	6	Meat	10·0	—	—	0·786	77·0	3·0	20·0
	„ 22	3–5	Meat	4·0	—	—	0·820	68·0	17·0	15·0
	1914									
	Feb. 2	3	Butter	—	—	50·0	0·733	9·5	7·0	84·0
	„ 9	3	P.b.	3·0	60·0	—	0·900	17·0	75·0	8·0
	„ 13	3	P.b.	3·0	60·0	—	0·855	20·0	60·0	20·0
	„ 24	5–7	Meat	8·0	—	—	0·802	62·0	11·0	27·0
	Mar. 5	7	Meat Erept.	9·0	—	—	0·803	74·0	7·5	18·5
	„ 11	3	C. and Pal.	—	—	—	0·767	17·0	13·0	70·0
	„ 18	4	C. and Pal.	—	—	—	0·744	29·0	0·0	71·0
	„ 19	1	C. and C.L.O.	—	—	—	0·750	33·0	0·0	67·0
	„ 20	3	C. and C.L.O.	—	—	—	0·707	10·0	0·0	90·0
	„ 23	4	C. and Pal.	—	—	—	0·730	20·0	0·0	80·0
VIII	June 22	2	Bread and Meat	—	—	—	0·921	34·0	66·0	0·0
	July 2	3	P.b.	—	—	—	0·861	30·0	43·0	27·0

When the animals are diabetic calculations of the percentage energy distribution become more complicated, owing to the fact that part of the protein which normally makes up the protein quota of the total metabolism is lost to the organism as excreted sugar. Beside this sugar formation from protein which we attempt to allow for in the calculations of percentage energy distribution, other processes may occur, such for example as the formation of sugar from fat or of the acetone bodies, which may still further complicate such energy estimations. The calculations, therefore, even after allowing for sugar formation from protein, are more a rough indication than a quantitative index, because of complexities for which one cannot allow. We have, however, thought that some indication would be afforded of the

character and extent of the carbohydrate and fat portions. In order, therefore, to get some idea of the energy distribution, a side calculation has been made allowing for sugar formation from protein based upon the observed D : N ratio. If one allows for the sugar formation from protein, as in the following example, the respiratory quotient of the remaining protein carbon dioxide and oxygen can be easily calculated. As the dextrose-nitrogen ratio increases from 2·6 to 4·0 the respiratory quotient decreases from 0·725 to 0·620. It can be readily seen, therefore, how much a perversion of protein metabolism with, say, a D : N ratio of 3·8 would contribute to the lowering of the respiratory quotient of diabetics. Such an example is:

Dog IV. Mar. 3, 1914.

Fasting 48 hours.
Total N 5·10. D : N 2·9

	CO_2	O_2	
Nitrogen per hour 0·210	1026	1268	Protein to sugar correction
Glucose „ 0·60	444	444	
	582	824	

	CO_2	O_2	R.Q.
Total figures observed	3643	5146 ⟶	0·700
Protein minus glucose	582	824	
	3061	4322 ⟶	0·707

In this example the protein percentage is 16 % and the remaining 84 % has a quotient indicating fat combustion. A number of such calculations have been made and are given in the tables. The percentage part in the total metabolism played by protein is increased in diabetes according to our observations. The protein percentage ranges from 16 to 32 % on the first and second days after depancreatisation, with an average of 25 %.

Table VII (p. 200) contains a summary of the figures for percentage energy distribution in the diabetic dogs in the fasting and digesting state. This table contains a number of observations which may be compared with those in Tables III and IV. In the digestion of a protein meal the level of the respiratory quotient is much lower in the diabetic animals than in the normal, and the allowance for sugar formation from protein in most cases indicates that the energy is distributed to protein and fat and that the oxidation of the fat portion is complete.

In Table V are given the results of calculations of the percentage energy distribution in diabetic dogs on the early days after operation. The protein portion is reckoned on the total 24 hours nitrogen. It will be noted that this protein percentage varies greatly, but the usual figure is distinctly higher

than the normal percentage when no protein has been given for periods of 48 to 60 hours. The excessively high figure in the case of Dog VII after partial depancreatisation is surprising. In this dog there was an extremely slight glycosuria and in the calculation no allowance is made for sugar form-

TABLE V.

No. of dog	Depanc.	Days after operation	Prot.	Carb.	Fat
I	Total	1st	32·0	4·0	64·0
		2nd	30·0	0·0	70·0*
III	Total	1st	24·0	0·0	76·0*
IV	Total	1st	16·0	0·0	84·0
V	Total	2nd	18·0	0·0	82·0*
VI	Total	2nd	21·0	10·0	69·0
VII	Partial	2nd	30·0	10·0	60·0
		3rd	24·0	7·0	69·0
VIII	Partial	2nd	62·0	0·0	38·0*
	Total	1st	52·0	2·0	46·0
	Total	2nd	19·0	2·0	79·0

ation from protein, since practically no sugar was excreted, but the results indicated the probability of such formation and also that the sugar, if formed, was unutilised since the quotient of the remainder after deducting the protein portion is far below that of the combustion of fat. The full figures of this observation will be given:

Dog VII. May 20. 72 *hours since food was given.*

	CO_2 1 hour	O_2 1 hour	R.Q.
Total N 9·5 g. Total sugar excretion 2·25	2889	3894	→ 0·742
Protein 1 hour N 0·40	1954	2415	
Carbohydrate and fat ..	935	1479	→ 0·632
Protein 62 %.	Fat 38 % (incomplete?).		

With this dog it will be noted that two months later, on the first day after the total depancreatisation, the protein figure is again very high, but when one allows for the sugar formation from protein on the basis of the observed D : N ratio, one sees that the combustion of the fat portion is complete.

Dog VII. July 18. 44 *hours since any food was given.*

	CO_2	O_2	CO_2	O_2	R.Q.
Total N 9·8 g. N 0·408	1994	2463	2198	3084	→ 0·712
D : N 2·8, Gl. 1·14	847	847	1147	1616	
	1147	1616	1051	1468	→ 0·715
Protein 52·0.	Carbohydrate 2·0.		Fat 46·0.		

It therefore appears very probable that in the first instance sugar was being formed from protein, that this sugar was not being utilised to any great extent and also that it was not being excreted. We obtained no striking evidence of incomplete combustion of fat in an increase of acetone bodies excreted, which indicates that the conclusions are justified.

(*b*) *Fat.* The percentage energy distribution to fat in the normal animals in a fasting condition can be seen from the results in Table VIII to be uniformly high. Feeding the animals upon fat alone was not carried out with success, but with large amounts of fat fed with protein the fat takes a predominant part in the total metabolism (Table IV, p. 186) with a resultant lowering of the respiratory quotient. We obtained no evidence of incomplete combustion of fat in the normal animals. Great interest centres around the question of the combustion of fat in diabetes. In Table V the figures for fat marked (*) are cases in which the combustion of fat was apparently incomplete as the remainder quotient after deducting for protein was lower than the normal fat quotient. We find no parallelism between this apparent incomplete combustion of fat and the excretion of acetone bodies. The latter increase steadily throughout the course of the diabetes and frequently show fluctuations which cannot be correlated with any marked change in the respiratory quotient. The course of excretion of acetone bodies seems to bear a closer relation, though not very close, to the protein breakdown. Also in the cases in which fat was fed to the animals the excretion of fat in the faeces never indicated a deficient absorption of the fat fed. The fats, therefore, as far as these observations upon dogs indicate, are apparently well dealt with by the diabetic organism. In some cases evidence of incomplete combustion of fat appears. In the greater number of cases the low respiratory quotient of diabetic dogs appears to be caused, not so much by a perversion of the fat metabolism as by the disturbance of the normal combustion of protein.

(*c*) *Carbohydrate.* The extent to which carbohydrate is oxidised in diabetic as compared with normal dogs is, of course, the question which is the most important to us at present. It is necessary, therefore, to find out what percentage part of the total metabolism is taken by carbohydrate in the normal animal under various conditions.

In the normal animal it is obvious that the amount of sugar used in the oxidations will depend firstly upon the supply available. Thus in the fasting animal the percentage distribution to carbohydrate may vary from 0 to 30 % depending upon the diet, whether rich or poor in carbohydrate, and the carbohydrate storage in the tissues. Table III (p. 185) contains the figures

calculated as described above from a series of normal fasting observations.

Dog III.

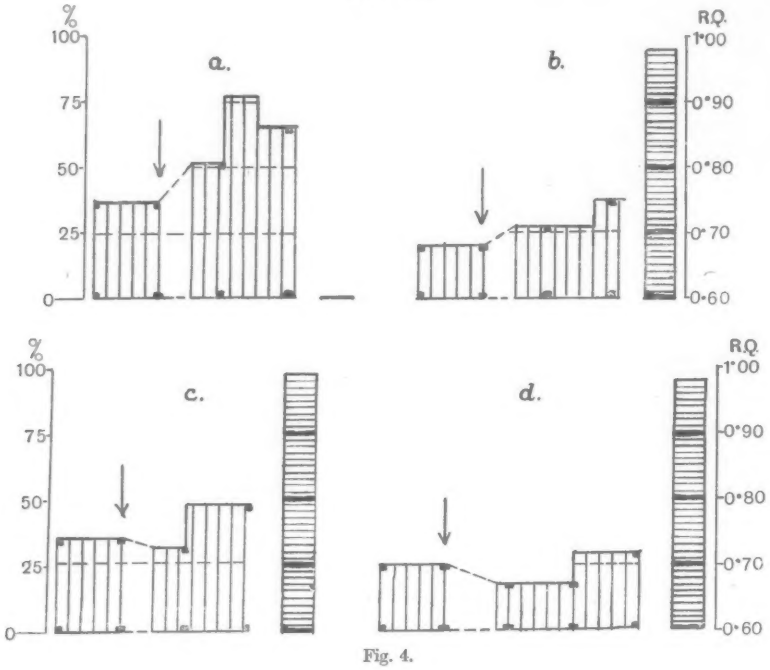


Fig. 4.

Dog IV.

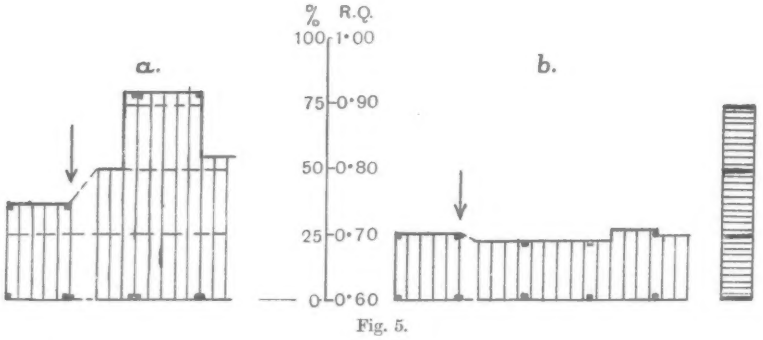


Fig. 5.

When glucose or fructose is given to normal animals the utilisation is shown by a marked rise of the respiratory quotient. Within an hour after

Dog VII.

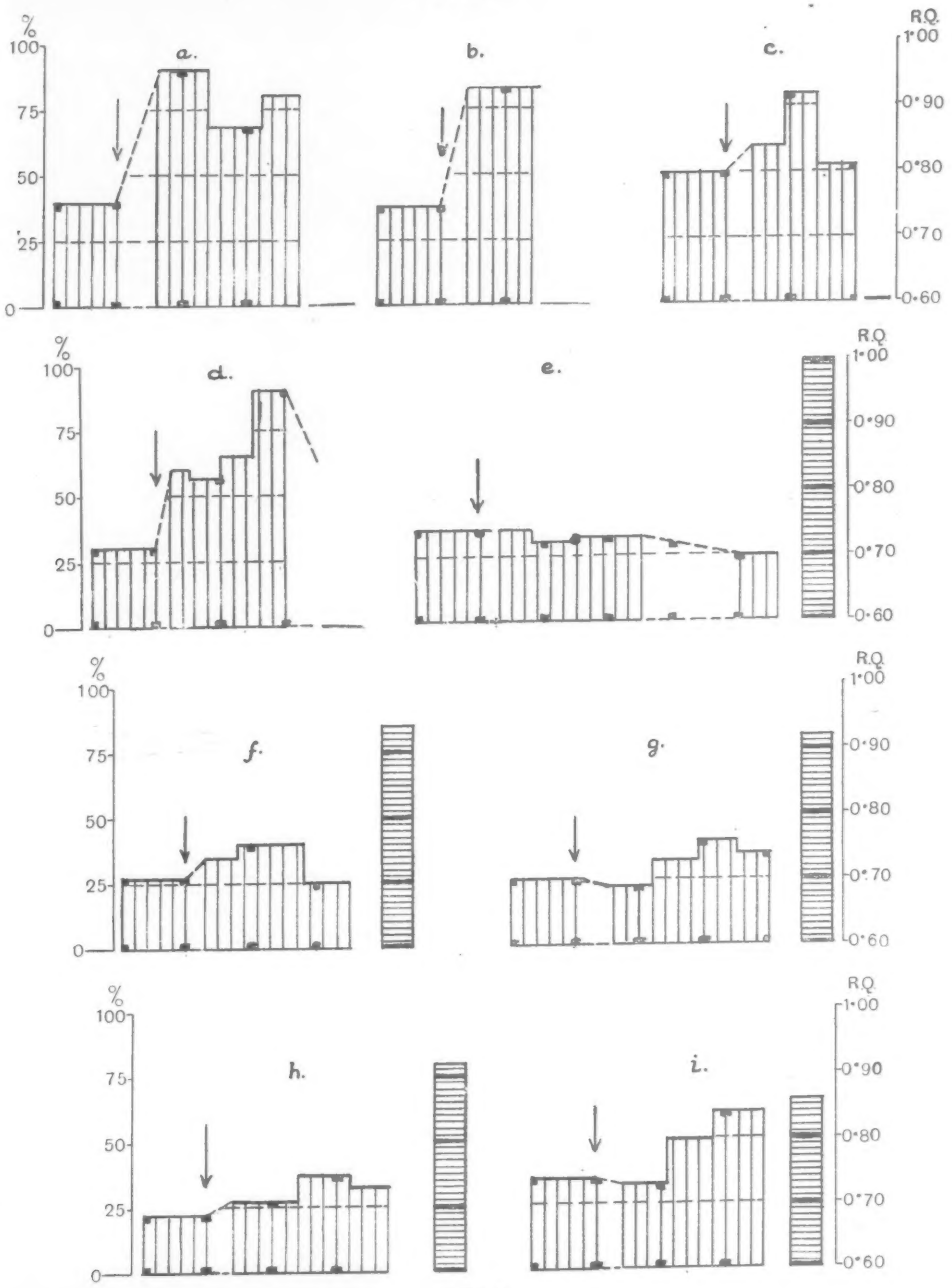
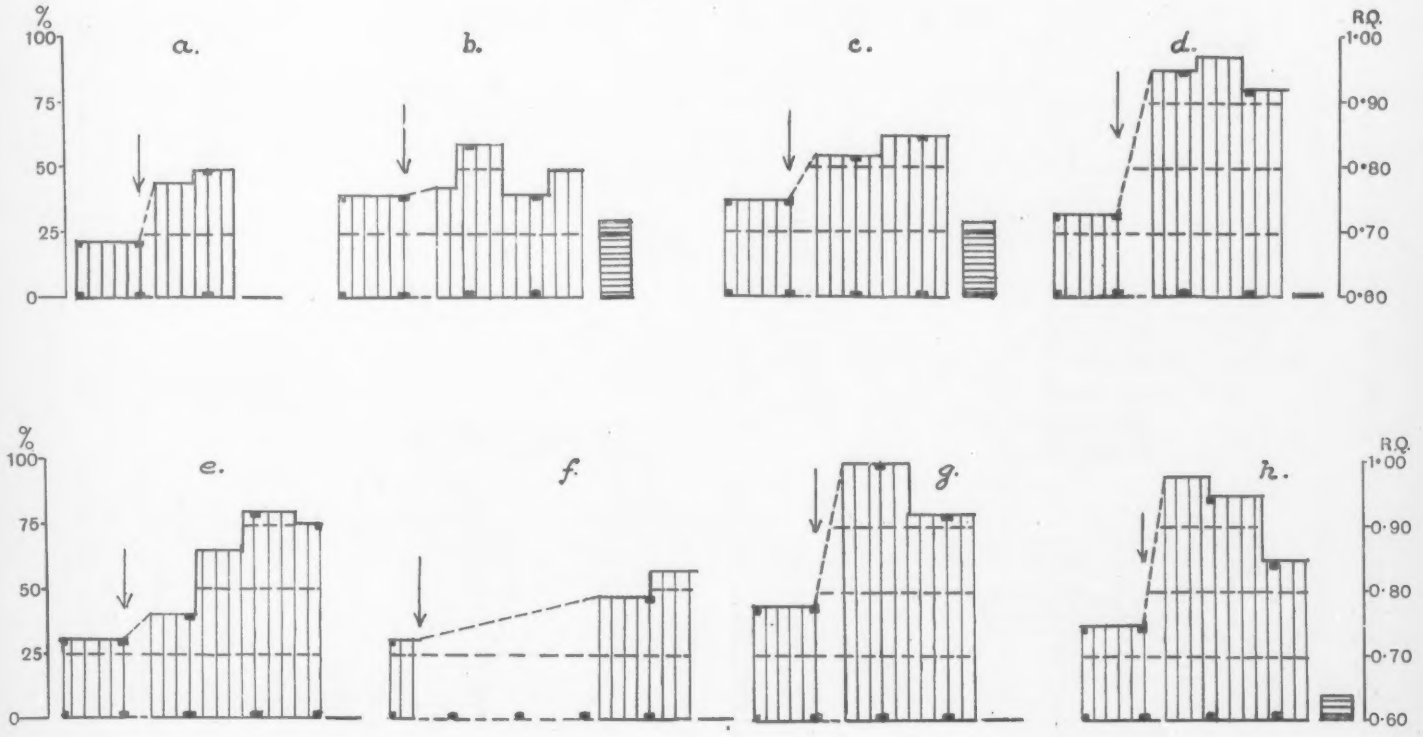


Fig. 6.

Dog VIII.



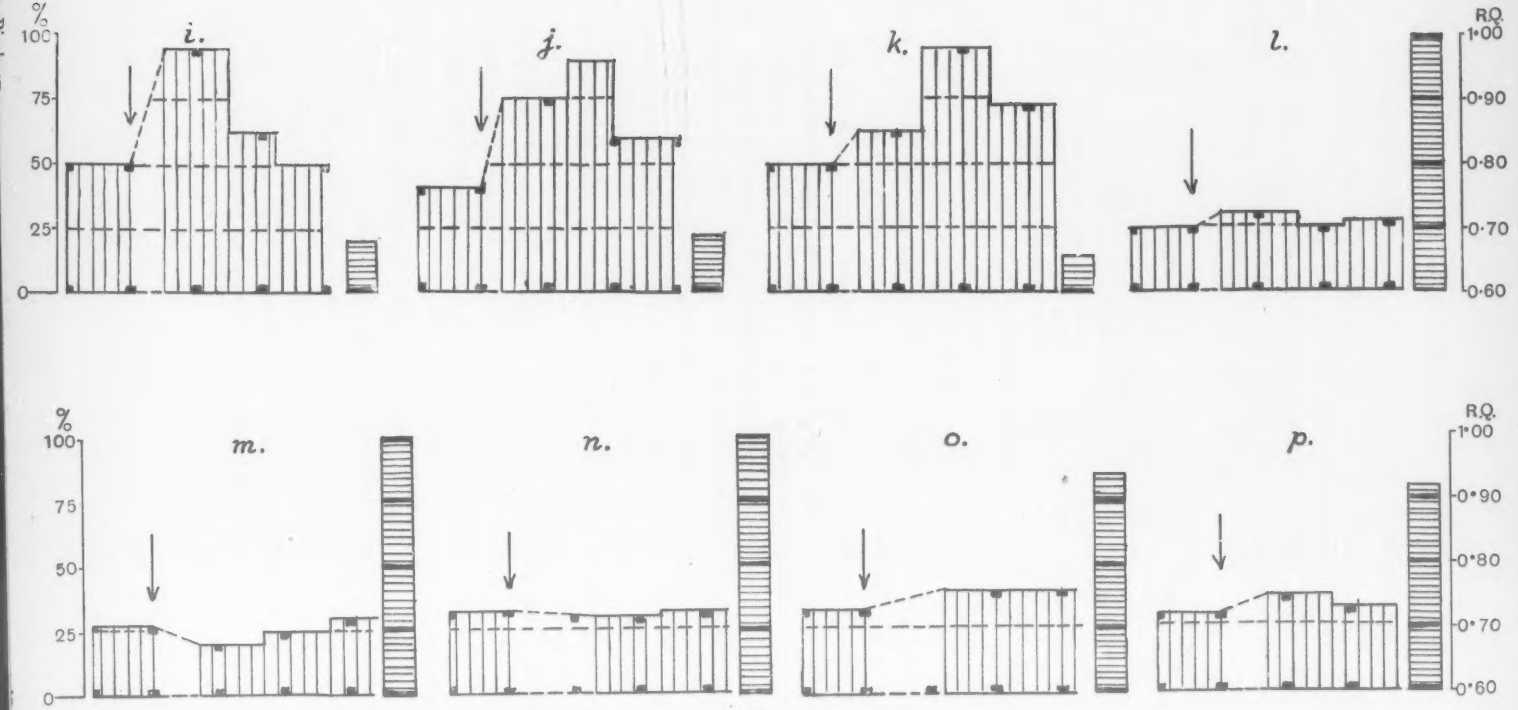

%
100
75
50
25
0
i.
j.
k.
l.
R.Q.
1·00
0·90
0·80
0·70
0·60
%
100
75
50
25
0
m.
n.
o.
p.
R.Q.
1·00
0·90
0·80
0·70
0·60

Fig. 7.

the sugar has been taken the quotient has risen to 0·90 to 1·00 and for three or more hours remains between 0·80 and 0·90, gradually returning to its original level. If calculations are made of the percentage energy distribution, the carbohydrate portion increases up to 80 to 90 % (see Table VI). The following is a protocol of an observation before and after the giving of glucose:

Dog IV. Feb. 27, 1914.

Period No.	Duration mins.	CO_2 cc. per min.	O_2 cc. per min.	R.Q.	Protein	Carbohydrate	Fat
I	50	54·1	72·2	0·749	15·0[1]	10·0	75·0
		30 g. glucose given 20 mins. before II period.					
II	20	85·2	106·0	0·803	15·0	27·0	68·0
III	40	84·6	92·2	0·917	15·0	67·0	18·0
IV	30	77·7	84·5	0·919	15·0	70·0	15·0
V	30	71·5	87·6	0·816	15·0	35·0	50·0

[1] Assuming protein to be 15 %.

With the doses of glucose given (20 to 40 g.) no sugar appeared in the urine of normal dogs. In the normal rise of the quotient after giving sugar the minute figures for carbon dioxide and oxygen both increase, but the amount of the carbon dioxide increases far more than the oxygen intake. The rise in the respiratory quotient is due to an increase in the output of CO_2.

In Figs. 4, 5, 6 and 7 (pp. 190–193) a series of observations before and after the giving of sugar are plotted as curves. The curves hatched vertically represent the levels of the respiratory quotient during the successive periods. Five of the vertical lines represent a duration of one hour. The arrow marks the time at which the sugar was given. The second part of the curve is hatched horizontally and shows the percentage of the sugar which was recovered from the urine, over and above the amount of sugar corresponding to the nitrogen excreted on the basis of the D : N ratio of the day before and after. The protocols corresponding to Figs. 4, 5, 6, 7 will be found in Table VI. In the normal animal it is seen from these results that the rise of the respiratory quotient after sugar is a well-marked one. If we compare these results with the effect of giving sugar to the diabetic animals, two important points are brought out:

(i) The rise of the respiratory quotient in the diabetic animals is diminished in all cases, and frequently fails to appear.

(ii) The level of the quotient after giving sugar corresponds very closely to the percentage excretion of the sugar given. If all the sugar is excreted the quotient shows little or no rise, and if only a slight excretion occurs, as in the case of Dog VIII, the increase in the respiratory quotient approaches the normal.

TABLE VI. *Respiratory quotient, minute figures for CO_2 and O_2 and percentage energy distribution before and after sugar administration.*

No. of animal	Date	Period No.	Duration min.	Sugar	CO_2 cc. p. m.	O_2 cc. p. m.	R.Q.	% energy distribution: Protein	Carbohydrate	Fat	Notes
	1914										
III	Feb. 11	I	60		66·75	89·5	0·745	15·0	9·0	76·0	Normal.
	a.			Gluc. 30 g.							
		II	30		76·0	94·0	0·806	—	—	—	
		III	30		67·26	75·9	0·908	15·0	65·0	20·0	
		IV	35		68·5	79·43	0·862	—	—	—	Depancreatised Feb. 16.
	Feb. 18	I	60		57·86	85·5	0·680	24·5	0·0	75·5	D : N = 3·2.
	b.			Gluc. 30							
		II	70		55·5	78·3	0·713	26·8	3·2	70·0	
		III	20		59·8	79·2	0·754	—	—	—	Sugar excreted = 95 %.
	Feb. 19	I	40		54·2	73·0	0·740	25·0	11·0	64·0	D : N = 3·4.
	c.			Gluc. 20							
		II	20		59·5	82·1	0·724	26·0	20·0	54·0	
		III	30		52·6	66·6	0·789				
		IV	30		77·5	98·1	0·790	—	—	—	Sugar excreted = 95 %. Dog very restless.
	Feb. 21	I	60		48·05	67·9	0·699	21·0	4·0	75·0	D : N = 3·4.
	d.			Gluc. 20							
		II	40		49·3	74·4	0·672	20·0	0·0	80·0	
		III	30		48·5	72·3	0·671				
		IV	60		58·15	81·3	0·723	18·0	6·5	75·5	Sugar excreted = 95 %.
IV	Feb. 27	I	50		66·85	89·1	0·750	—	—	—	
	a.			Gluc. 30							
		II	20		85·2	106·0	0·803	—	—	—	
		III	40		84·6	92·2	0·917	—	—	—	
		IV	30		77·7	84·5	0·920	—	—	—	
		V	30		71·5	87·6	0·820	—	—	—	Depancreatised Mar. 2.
	Mar. 6	I	60		51·05	72·4	0·705	15·0	2·0	83·0	D : N = 3·8.
	b.			Gluc. 30							
		II	43		58·4	84·0	0·696	—	—	—	
		III	41		57·3	83·7	0·685	—	—	—	
		IV	40		56·0	81·3	0·688	13·0	0·0	87·0	
		V	40		55·0	76·7	0·716	—	—	—	
		VI	32		55·97	79·03	0·708	15·0	2·0	83·0	Sugar recovered 75 %.
	1913										
VII	Dec. 3	I	45		69·34	90·3	0·760	12·0	17·0	71·0	Normal.
	a.			Gluc. 30							
		II	45		83·55	86·95	0·96	12·7	82·0	5·3	
		III	45		67·98	77·98	0·87	—	—	—	
		IV	45		73·24	79·95	0·92	—	—	—	
	Dec. 5	I	45		68·50	91·0	0·750	13·0	13·0	74·0	
	b.			Fruct. 30							
		II	60		95·55	102·98	0·930	11·0	73·0	16·0	

TABLE VI—*Continued*

No. of animal	Date	Period No.	Duration min.	Sugar	CO_2 cc. p. m.	O_2 cc. p. m.	R.Q.	% energy distribution: Protein	Carbohydrate	Fat	Notes
	1914										
VII	Jan. 15	I	30		64·3	79·77	0·807	15·0	30·0	55·0	
	c.			Gluc. 10							
		II	30		64·8	77·3	0·840	—	—	—	
		III	30		73·8	80·4	0·920	15·0	70·0	15·0	
		IV	30		68·0	84·5	0·805	—	—	—	
	May 14	I	40		52·8	73·0	0·720	15·0	0·0	85·0	
	d.			Gluc. 20							
		II	20		74·8	89·35	0·837	—	—	—	
		III	30		68·7	83·5	0·822	—	—	—	
		IV	30		65·5	75·86	0·860	—	—	—	
		V	30		70·94	73·4	0·966	15·0	85·0	0·0	Partially depancreatised May 18.
	May 28	I	60		46·1	61·9	0·743	32·0	13·0	55·0	D : N = 2·9.
	e.			Gluc. 20							
		II	33		49·0	65·8	0·744	—	—	—	
		III	40		51·77	71·7	0·720	—	—	—	
		IV	60		55·5	75·5	0·730	28·0	10·0	62·0	Sugar excreted 100 %.
		V	32		46·0	66·4	0·691	30·0	0·0	70·0	4 hrs. after the sugar.
	June 3	I	60		40·55	56·4	0·718	14·0	0·0	86·0	D : N = 3·1.
	f.			Gluc. 20							
		II	30		39·0	53·0	0·736	—	—	—	
		III	60		47·9	63·17	0·758	17·0	12·0	71·0	
		IV	40		44·52	63·55	0·700	15·0	0·0	85·0	85 % of sugar excreted.
	June 8	I	60		39·9	56·94	0·700	14·0	4·0	82·0	D : N = 3·7.
	g.			Fruct. 20							
		II	40		52·3	76·2	0·686	20·0	5·0	75·0	
		III	43		46·16	62·8	0·734				
		IV	37		44·13	58·1	0·759	23·0	20·0	57·0	
		V	30		47·3	64·1	0·738				Sugar excreted 80 %.
	June 13	I	50		38·6	56·90	0·678	20·0	0·0	80·0	D : N = 3·4.
	h.			Gluc. 20							
		II	60		37·33	52·16	0·715	2·0	5·0	93·0	
		III	45		43·06	57·4	0·749	2·0	15·0	83·0	
		IV	35		42·0	57·0	0·736	—	—	—	Sugar excreted 80 %.
	June 24	I	60		34·35	46·32	0·741	20·0	17·0	63·0	D : N = 3·8.
	i.			Gluc. 20							
		II	40		53·8	73·9	0·727	—	—	—	
		III	40		46·17	58·0	0·796	—	—	—	
		IV	40		39·05	46·6	0·837	23·0	40·0	37·0	Sugar excreted 65 %.
	June 18	I	80		54·81	77·4	0·707	40·0	7·0	53·0	D : N = 3·5. Intravenous injection.
				Gluc. 20							
		II	35		54·0	77·4	0·698	—	—	—	
		III	35		53·8	76·1	0·706	—	—	—	
		IV	30		55·9	79·0	0·708	39·0	5·0	56·0	

TABLE VI—*Continued*

No. of animal	Date	Period No.	Duration min.	Sugar	CO_2 cc. p. m.	O_2 cc. p. m.	R.Q.	% energy distribution: Protein	Carbohydrate	Fat	Notes
	1914										
VIII	May 16	I	30		53·26	77·1	0·690	10·0	0·0	90·0	Normal.
	a.			Gluc. 10							
		II	35		62·1	79·3	0·782	—	—	—	
		III	35		55·7	70·0	0·796	10·0	30·0	60·0	
	May 25	I	60		46·9	61·8	0·758	15·0	14·0	71·0	Partial depancreatisation on May 18.
	b.			Gluc. 20							Slight glycosuria.
		II	20		52·4	67·8	0·773	—	—	—	
		III	40		48·25	57·25	0·842				
		IV	40		50·9	66·5	0·765	15·0	28·0	57·0	Total sugar exc. = 11·0 g.
		V	30		54·66	68·4	0·798				
	May 29	I	60		43·75	58·4	0·748	15·0	10·0	75·0	
	c.			Gluc. 20							
		II	60		48·66	59·36	0·820	—	—	—	
		III	60		52·08	61·5	0·846	15·0	43·0	42·0	Total sugar exc. = 5·9 g.
	June 5	I	35		35·6	48·57	0·733	15·0	6·0	79·0	No glycosuria.
	d.			Fruct. 20							
		II	42		54·05	56·9	0·949	—	—	—	
		III	40		50·4	51·7	0·974	15·0	85·0	0·0	
		IV	40		51·9	56·3	0·921	—	—	—	No sugar excreted.
	June 9	I	40		37·3	51·6	0·723	15·0	0·0	85·0	
	e.			Gluc. 20							
		II	40		42·75	56·0	0·763	—	—	—	
		III	40		50·4	58·2	0·864	—	—	—	
		IV	45		49·66	53·8	0·922	15·0	70·0	15·0	
		V	20		49·6	54·8	0·904	—	—	—	No sugar excreted.
	July 1	I	60		39·3	50·66	0·777	15·0	20·0	65·0	
	g.			Gluc. 20							
		II	60		54·9	52·85	1·03	15·0	85·0	0·0	No sugar excreted.
		III	60		49·95	54·3	0·920				
	July 4	I	45		38·44	51·33	0·749	15·0	9·0	76·0	Thyroid administered.
	h.			Gluc. 20							
		II	40		52·4	53·25	0·984	10·0	90·0	0·0	
		III	50		62·6	66·2	0·946	—	—	—	
		IV	40		46·3	54·37	0·851	—	—	—	Total sugar exc. = 2·1 g.
	July 9	I	60		47·57	58·95	0·806	15·0	26·0	59·0	
	i.			Gluc. 20							
		II	60		65·66	67·08	0·978	15·0	85·0	0·0	
		III	40		52·8	62·17	0·849	—	—	—	
		IV	40		49·75	62·17	0·800	15·0	25·0	60·0	Total sugar exc. = 4·3 g.
	July 14	I	60		38·55	50·47	0·764	15·0	16·0	69·0	
	j.			Gluc. 20							
		II	60		44·66	49·65	0·900	—	—	—	
		III	40		49·0	51·1	0·958	15·0	80·0	5·0	
		IV	50		49·36	58·68	0·841	—	—	—	Total sugar exc. = 4·8 g.

TABLE VI—*Continued*

No. of animal	Date	Period No.	Duration min.	Sugar	CO_2 cc. p. m.	O_2 cc. p. m.	R.Q.	% energy distribution: Protein	Carbohydrate	Fat	Notes
	1914										
VIII	July 16	I	60		37·65	46·7	0·806	15·0	29·0	56·0	Thyroid stopped.
	k.			Gluc. 20							
		II	60		39·6	46·5	0·852	—	—	—	
		III	60		44·77	45·73	0·979	10·0	90·0	0·0	
		IV	60		41·9	47·2	0·887	—	—	—	Total sugar exc. 3·3 g. Totally depancreatised, July 17.
	July 20	I	60		37·39	53·4	0·700	36·0	0·0	64·0	D : N = 3·0.
	l.			Gluc. 20							
		II	71		39·3	54·8	0·717				
		III	40		40·35	57·45	0·702	35·0	1·0	64·0	Sugar excreted 100 %.
		IV	50		38·36	53·92	0·711				
	July 22	I	60		37·63	53·01	0·709	20·0	4·0	76·0	D : N = 3·1.
	m.			Gluc. 20							
		II	60		38·25	55·93	0·684	—	—	—	
		III	60		40·2	57·03	0·705	20·0	0·0	80·0	
		IV	40		38·8	53·4	0·720	—	—	—	Sugar excreted 100 %.
	July 24	I	60		38·06	52·13	0·730	20·0	10·0	70·0	D : N = 3·1.
	n.			Gluc. 20							
		II	62		38·0	52·64	0·721	—	—	—	
		III	60		39·16	53·66	0·730	20·0	7·0	73·0	Sugar excreted 100 %.
	July 28	I	60		36·13	49·26	0·733	20·0	12·0	68·0	D : N = 3·1.
	o.			Fruct. 20							
		II	60		38·76	50·8	0·763				
		III	60		39·1	51·53	0·758	20·0	20·0	60·0	Sugar excreted 85 %.
	July 31	I	50		38·88	54·0	0·720	15·0	7·0	78·0	D : N = 3·1.
	p.			Gluc. 20							
		II	60		44·02	58·73	0·749				
		III	60		42·33	57·95	0·730	14·0	13·0	73·0	Sugar recovered 80 %.

When the respiratory quotient shows no rise, as for instance with Dog IV *b*, Fig. 5, the minute figures for carbon dioxide and oxygen will be found to show a parallel increase; see protocol IV *b* in Table VI.

In some of the totally depancreatised dogs in the later stages, it was observed several times that the respiratory quotient might rise noticeably, simulating the elevation after giving sugar. The cause of this rise was found to be a diminution in the oxygen intake with a very constant carbon dioxide output. The following is an instance:

Dog I.

Feb. 4th, 1913.	CO_2	O_2	R.Q.
Period I. 40 mins.	45·67	62·72	0·728
Given 15 g. glucose			
Period II. 30 mins.	45·60	60·2	0·750
Period III. 42 mins.	46·70	54·0	0·863
Feb. 5th. No sugar given			
Period I. 30 mins.	39·6	63·6	0·624
Period II. 45 mins.	39·4	46·7	0·844
Period III. 40 mins.	39·7	53·5	0·742

This animal was in a very bad condition when these observations were made. This condition may lead to confusing results and the quotient is of no value unless the minute figures are analysed. The variations in the taking up of oxygen may be due to a periodic type of breathing, but we regard the duration of the observation as long enough to equalise any such effect, and think that the blood changes must be responsible for the continual decrease in the taking up of oxygen observed. The respiration rate has been observed to vary from 15 to 43 per minute without any irregularity in the oxygen intake—no measurement of the depth of respiration was made. Several instances such as the above have been observed when the animals showed great circulatory and muscular feebleness, and were so quiet in the apparatus that the possibility of errors in the oxygen measurement is excluded.

As is seen in Figs. 5, 6 and 7 the rise of the respiratory quotient caused by sugar is absent in observations shortly after depancreatisation and later may occur in a very diminished form. In the totally depancreatised Dog III some evidence of sugar utilisation is present shortly after the operation, but the last observation (Fig. 4) indicates practically no utilisation of sugar. Dog IV when diabetic showed no indication that sugar was being used (Fig. 5 *b*). The series of observations shown in Fig. 7 on Dog VIII gives a very good comparison of the partially and totally depancreatised state. In this figure *b* and *c* are made shortly after the partial depancreatisation and show less marked increase of the R. Q. than the succeeding observations when practically none of the sugar was recoverable from the urine. The dog apparently recovered its normal power of utilising glucose. The observations *l*, *m*, *n*, *o* and *p*, after the remnant of pancreas was removed, show very little evidence of sugar utilisation. Dog VII shortly after partial depancreatisation gave no indication of sugar utilisation, as is seen in Fig. 6 *e*. Later the graft apparently established its disturbed circulatory conditions and a return of the power of oxidising sugar is evident from the rise of the respiratory quotient (Fig. 6 *h*, *i*).

The percentage energy distribution to carbohydrate has been seen to vary in the normal fasting condition and after the giving of sugar to constitute a large part, 80 to 90 %, of the total metabolism. In such calculations in the fasting state of diabetic animals the figures, after allowance for sugar formation from protein, indicate that a small part is taken by carbohydrate in some cases. The results will be found in Table VII. Whether this is an apparent or real utilisation of carbohydrate can be seen to depend upon the accuracy with which the sugar formation from protein can be estimated from the D : N ratio observed. Thus, if too great an amount of sugar is taken to correspond with

TABLE VII.

Respiratory quotients and percentage energy distribution in diabetic animals.

No. of animal	Date	Hours after	Diet	N	Carbohydrate	Fat	R.Q.	% energy dist. Protein	Carbohydrate	Fat	D/N	Notes
I	Jan. 31	40	Meat	12·0	—	—	0·717	32·2(1)	4·0	64·0	3·1	
	Feb. 1	64	Meat	12·0	—	—	0·691	30·0	0·0	**70·0**(2)	3·0	
	„ 2	24	Meat	8·0	—	—	0·688	29·0	0·0	**71·0**	2·7	
	„ 3	20	Meat	4·0*	—	—	0·708	24·0	3·0	73·0	3·2	* Intake less—dog vomiting.
	„ 4	18	Meat	4·0*	—	—	0·720	9·0	9·0	82·0	3·2	
III	Feb. 17	41	Meat	4·0	—	—	0·692	24·0	0·0	**76·0**	2·7	
	„ 18	3	M. and P.	4·0	—	—	0·680	24·5	0·0	**75·5**	3·2	
	„ 19	3	M. and P.	4·0	—	—	0·740	25·0	11·0	64·0	3·4	
	„ 20	7	M. and P.	4·0	—	—	0·680	41·0	0·0	59·0	3·8	
	„ 21	18	M. and P.	4·0	—	—	0·690	21·0	4·0	75·0	3·4	
	„ 23	19	M. and P	4·0	—	—	0·705	22·0	3·0	75·0	3·4	
IV	Mar. 3	48	Meat	4·0	—	—	0·700	16·0	0·0	84·0	2·9	
	„ 4	5	M. and P.	5·5	—	—	0·691	21·0	0·0	79·0	3·5	
	„ 6	20	M. and P.	4·0	—	—	0·705	15·0	2·0	83·0	3·8	
V	Mar. 4	67	M. and P.b.	8·0	50·0	—	0·677	18·0	0·0	**82·0**	3·0	
	„ 5	4	M. and P.	4·0	—	—	0·700	36·0	0·0	64·0	3·0	
	„ 7	36	M. and P.	4·0†	—	—	0·670	10·0	0·0	**90·0**	4·0	† Dog vomiting.
VI	July 29	60	Meat	8·0	40·0†	—	0·731	21·0	10·0	69·0	3·1	Fructose day before.
VII	May 20	72	Meat	8·0	—	—	0·733	30·0	10·0	60·0	2·6	Milk 100 cc. 19 hrs. before.
	„ 21	24	Milk	1·0	10·0	6·0	0·725	24·0	7·0	69·0	2·6	
	„ 23	18	M. and P.	8·5	—	—	0·676	34·0	0·0	**66·0**	3·2	
	„ 27	17	M. and P.	8·5	20·0	—	0·715	42·0	3·0	55·0	2·8	Glucose day before.
	„ 28	18	M. and P.	8·5	—	—	0·743	32·0	13·0	55·0	2·9	
	„ 29	18	M. and P.	7·5	20·0	—	0·693	36·0	0·0	**64·0**	2·9	Glucose day before.

	June	1	24	M. and P.	7·5	—	—	0·706	30·0		0·0	70·0	2·8	
	„	2	3	M. and P.	7·5	—	—	0·693	43·0		0·0	**57·0**	2·8	
	„	3	22	M. and P.	7·5	—	—	0·710	14·0	N.O.	0·0	86·0	3·1	
	„	8	24	M. and P.	9·5	—	—	0·700	14·0	N.O.	4·0	82·0	3·7	
	„	13	18	M. and P.	10·0	—	—	0·693	20·0	N.O.	0·0	80·0	3·4	
	„	18	3	Cas. and P.	6·0	—	—	0·707	40·0	N.O.	7·0	53·0	3·5	
	„	24	18	Gel. and P.	6·0	—	—	0·741	20·0	N.O.	17·0	63·0	3·8	
	„	26	24	Cas. and P.	6·0	—	—	0·696	19·0		0·0	81·0	3·7	
VIII	May	20	72	Meat. Milk 100 cc. day before	8·0	—	—	0·742	62·0[1]		0·0	**38·0**	—	Total sugar 2·25.
	„	25	24	M. and P.	5·5	—	—	0·758	36·0		6·0	58·0	—	Total sugar 11·0.
	„	29	24	M. and P.	5·5	—	—	0·748	27·0		9·5	63·5	—	Total sugar 5·9.
	June	22	2	Bread and Meat	6·5	100·0	—	0·921	34·0		61·0	5·0	—	No glycosuria.
	„	29	18	P.b.	5·0	60·0	—	0·780	34·0		14·0	52·0	—	„ „
	„	30	18	Meat and P.b.	7·0	60·0	—	0·770	31·0		12·0	57·0	—	„ „
	July	2	3	P.b. and Meat	7·0	80·0	—	0·861	30·0		43·0	27·0	—	
	„	7	24	P.b. and Meat. Thyroid	7·0	60·0	—	0·785	28·0		18·0	54·0	—	
	„	8	19	„ „ „	7·0	60·0	—	0·809	29·0		26·0	45·0	—	
	„	9	18	„ „ „	7·0	60·0	—	0·806	35·0		22·0	43·0	—	
	„	18	44	M. and P.	11·0	20·0	—	0·712	19·0		2·0	79·0	2·8	
	„	25	18	„ „	11·8	20·0	—	0·710	20·0*		2·0	78·0	3·1	
	„	27	4	„ „	5·0	—	—	0·780	33·0		28·0	39·0	3·1	
	„	29	3	„ „	5·9	20·0	—	0·740	44·0		17·0	39·0	3·1	
	„	30	19	„ „	5·9	20·0	—	0·745	16·0[1]		15·0	69·0	3·1	
	„	31	18	„ „	11·8	—	—	0·720	15·0[1]		7·0	78·0	3·1	

[1] Protein metabolism has been calculated from 24 hours nitrogen excretion, except where marked * when the percentage has been assumed, and N.O. where the nitrogen excretion has been determined during the respiratory observation.

[2] Figures in black type denote that the calculation indicated an incomplete combustion of fat or other disturbance associated with abnormally low quotient.

the nitrogen excretion, deduction of the carbon dioxide and oxygen figures corresponding to protein minus sugar will indicate an apparent combustion of carbohydrate. In the majority of cases this amount of carbohydrate apparently utilised is quite small and frequently after sugar is given does not show any increase whatever, which is difficult of explanation if sugar is really being utilised.

4. *Results of Urine Analyses.*

Table VIII contains the results of the urine analyses as well as the total intake of food, balance sheets for nitrogen and phosphate, and general notes as to the condition of the animals.

(*a*) P_2O_5. It was thought that the increase of excreted phosphate indicated the tissue break-down in the diabetic animals. When a balance is estimated over a period, however, there is very little discrepancy between the intake and output. Dog VII showed a loss of 2·5 g. and Dog VIII a gain of 0·5 g. during the period.

(*b*) *Acetone bodies.* In Table VIII the figures for acetone represent the total acetone preformed and present as acetoacetic acid in the urine. The excretion of acetone bodies starts on an upward course soon after depancreatisation and shows many fluctuations from day to day with the general increase as the condition becomes more severe. We can find nothing definite to account for the considerable rise in the amount of acetone bodies in Dog VII on June 9 (Table VIII). 10 g. of sodium bicarbonate were administered on that day, but since on other occasions this does not cause a marked rise in acetone excretion, it may be due to something else. The question as to the chief source of the acetone bodies will be dealt with later in the report.

(*c*) *Sugar.* The sugar excreted in the diabetic urine was almost always dextro-rotatory and apparently glucose. When glucose was given to the diabetic dogs it was excreted in large amount. If the animals were catheterised after three to four hour periods, the rate of excretion of the sugar given could be ascertained. If we compare the sugar and nitrogen excretion after giving sugar (see Table IX) we see that the sugar is excreted in this case in large proportion during the first three hours after administration. On June 8 (Table IX) 20 g. fructose was administered and a small proportion of the sugar excreted was laevo-rotatory. On June 18 the animal received a protein meal before the first four hour period and the results illustrate the fact that the sugar is excreted before the nitrogen of the protein given.

(*d*) *Nitrogen excretion.* The total nitrogen balance shows a marked loss of nitrogen from the body in the early stages of diabetes. Later this loss becomes smaller and with a high protein intake equilibrium may be reached. Since the nitrogen loss is so marked in the early stages and the proportion of

TABLE IX. *Dog VII. Excretion of sugar and nitrogen after giving glucose and fructose.*

Date	No. of Period	Dur'n hours	Total glucose	Total nitrogen	D/N	N g. per hour	Notes
1914							
June 3	I	4	0·325	0·470	0·70	0·117	
	II	2·5	15·0	0·390	38·4	0·156	Given glucose 20 g. at beginning of period II.
	III	17·5	24·5	6·80	3·6	0·388	Meat and pancreas = 7·5 g. N at beginning of period III.
		24	39·8	7·66	5·1	—	Totals.
		24	38·5	7·14	5·3	—	Control on 24 hrs sample.
June 8	I	3	1·20	0·49	2·4	0·163	
	II	3	10·30[1]	0·80	13·0	0·266	Fructose 20 g. at beginning of period II.
	III	18	43·5	9·9	4·4	0·55	
		24	55·0	11·19	4·9	—	Totals.
		24	51·0	—	—	—	Control on 24 hrs sample. Glucose 48·0 Fructose 3·0
June 13	I	2	0·10	0·42	0·24	0·210	
	II	3	12·9	0·056	212·0	0·019	Glucose 20 g.
	III	19	35·7	9·13	3·9	0·480	Caseinogen and pancreas = 9·6 g. N at beginning of period III.
		24	48·7	9·61	5·0	—	Totals.
June 18	I	4	14·4	2·2	6·5	0·55	Caseinogen and pancreas = 6·0 g. N at beginning of period I.
	II	4	7·35	2·1	3·5	0·525	Intravenous injection 1·5 g. glucose at beginning of period II.
	III	16	21·8	7·0	3·1	0·44	Caseinogen and pancreas = 6·9 g. N at beginning of period III.
		24	43·5	11·3	3·8	—	Totals.
June 24	I	4	3·7	0·70	5·2	0·175	
	II	3	14·0	0·68	20·0	0·226	Glucose 20 g. at beginning of period II.
	III	17	41·7	11·82	3·5	0·695	Gelatin and pancreas = 12·9 g. N at beginning of period III.
		24	59·4	13·20	4·5	—	Totals.

[1] Dextrose 8·42. Fructose 1·88. Meat and panc. = 10·0 gm. N at beginning of period III.

sugar to nitrogen relatively small, and later increasing, the impression is given that the protein break-down in the earlier stages is resulting in the formation of sugar which accumulates in the blood to no purpose and overflows through the kidneys when a certain level is reached. This indication

has been mentioned above in connection with the increased protein metabolism in the partially depancreatised Dog VIII when practically no sugar was excreted. The total nitrogen excretion in the case of Dogs I and V is large directly after the total extirpation and decreases to a very small amount. This is accounted for by the fact that the animals on the fourth day after the operation vomited most of the food taken and the nitrogen excretion therefore indicates the break-down of the animal's own tissues.

(*e*) *Ammonia.* In Table VIII the ammonia is given both as g. and % of nitrogen. When expressed as percentage of total nitrogen the ammonia figure increases somewhat after total depancreatisation in the case of Dog III. In Dog VII the ammonia nitrogen percentage of the total nitrogen shows a slight increase when diabetes was produced. The increase is from 2 % to 3 % above the normal figure.

(*f*) *Urea.* As can be seen from Table VIII the percentage of urea nitrogen to total nitrogen is slightly lower in the diabetic animals than in the normal fed upon the same diet. In Dog VII, when normal and fed on meat and pancreas, the urea percentage was 85 to 90 % and when the animal was diabetic the percentage was slightly lower—80 to 87 %. Dog III before depancreatisation had a urea percentage of 90 % and after the operation 82 to 86 %.

(*g*) *Allantoin.* The average normal excretion of allantoin in the dog when meat is fed amounts to 0·2 to 0·5 g. N, or 2·4 % of the total nitrogen (see Table VIII). In the presence of sugar the allantoin estimation is upset unless special precautions are taken [Plimmer and Skelton, 1914, 2]. The allantoin figures for the diabetic dogs are of no value except in the case of Dog VIII. The allantoin nitrogen in this case increases up to 1·16 g. in the diabetic state and the percentage of the total nitrogen is about 10 %.

(*h*) *Uric acid.* As will be seen from the analyses figures for Dogs III and I, uric acid appears in the diabetic urine in estimable amounts and shows an increase as the diabetes progresses. In the normal dog the uric acid excretion is very small.

5. *Faeces.*

Analyses of the faeces for total nitrogen and fat and phosphate were carried out by Dr Cruickshank. The total nitrogen and P_2O_5 figures were used in estimating the balances to which reference has been made. The fat elimination in the faeces was generally small in normal and diabetic animals and the fats fed were evidently absorbed without difficulty from the intestine.

6. *Post-mortem Findings.*

General. The animals all showed great emaciation. The abdominal organs were examined, particularly the site of the pancreas on the duodenum. In the total depancreatisations the duodenum was removed and suspicious tissue examined histologically. No remnants of pancreatic tissue were found after the total extirpations. Portions of the heart and liver and in some cases suprarenals and thyroid were removed immediately after death.

Dog I. No signs of suppuration about wound or in the abdominal cavity. No remnants of pancreas present on the duodenum. Heart muscle flabby and pale. Liver pale. Suprarenals removed and histological appearance found to be normal.

Dog III. Some adhesions about the duodenal borders where pancreas had been removed. No pancreatic tissue found. No signs of suppuration or inflammation about wound or abdomen. Lymphatic nodes were enlarged, but this was noted at the operation. Artery walls very friable. This was noted also on many occasions by Evans, Patterson and Starling in their experiments on the heart-lung preparation of diabetic animals.

Dog VII. Some adhesions about the duodenum. Abdominal wound quite healed and closed. Graft was found to the right of the middle line under the skin of abdomen. It was hard, white and about one cm.^{2} in area. Blood supply evidently came to graft through a fold of omentum which was adherent to inner side of the abdominal wound. No remnants of pancreatic tissue in abdomen.

Histological appearance of graft. The tissue shows some, but not very marked evidence of degeneration. Acinous tissue in places looks quite normal. No islets of Langerhans could be identified with certainty.

Dog VIII. Adhesions between duodenum and colon and liver. Liver yellowish. Some small masses of tissue were removed from the duodenum and on histological examination were found to be masses of scar and lymphatic tissue—no pancreas remains found. No signs of suppuration about wound or abdomen.

Histological examination. Remnant of pancreas removed from the duodenum at operation II was found to show very little sign of degeneration. Acinous tissue was normal with some vacuolisation. Islets of Langerhans could be distinguished.

7. *Analysis of Organs for Glycogen and Fat.* (*Dr Cruickshank.*)

The glycogen estimations which have been carried out agree with the general results of Cruickshank's former investigation. The great increase of glycogen in the heart in the case of the partially depancreatised Dog VII is of interest, but more estimations are necessary before any conclusion can be

No. of animal	Organ	Glycogen %	Fat %	
VII	Heart	1·40	1·89	
	Liver	0·10	6·10	
VIII	Heart	0·924	2·6	
	Liver	0·025	10·7	
VI	Heart	0·50	2·37	
	Liver	0·20	5·84	
Normal	Heart	0·49	1·50	Average figures for
	Liver	4·0 to 7·0	2–3 %	normal animals.

drawn. The results have indicated that the heart muscle retains its power of adding to its glycogen store in diabetes and, perhaps because of the increased available amount of sugar, has actually a greater glycogen reserve than the normal. The increase in the percentage of fat in both cardiac and hepatic tissue is an evidence of the fat mobilisation in diabetes. A further analysis of the mobilisation of fat in diabetes would be of value and, it is hoped, will shortly be undertaken.

IV. Discussion and Summary.

Total Metabolism in Experimental Diabetes.

The results show a definite increase of total metabolism in pancreatic diabetes. This increase corresponds with the severity of the usual symptoms. In Dog VIII when partially depancreatised no increase was observed and the glycosuria was slight and transitory. In Dog VII partial removal of the pancreas resulted in a marked glycosuria and the average increase in the total metabolism is 9 %. In the totally depancreatised animals the increase was from 14 to 23 % and the type of diabetes very severe. Murlin and Kramer [1913] found an increase of metabolism amounting to 42 % in a depancreatised dog. Our figures represent an average throughout the course of the diabetes and the increase per cent. is found to rise during the diabetic period (Dog VIII) from 9 % to 38 %. The increase of total metabolism parallels the severity of the diabetes. Benedict and Joslin [1910, 1912] in their studies of diabetes in man calculated that the increase of

metabolism was from 15 % to 20 %. Lusk and others objected to this conclusion on the ground that the controls were unsuitable for comparison

Dog VII.

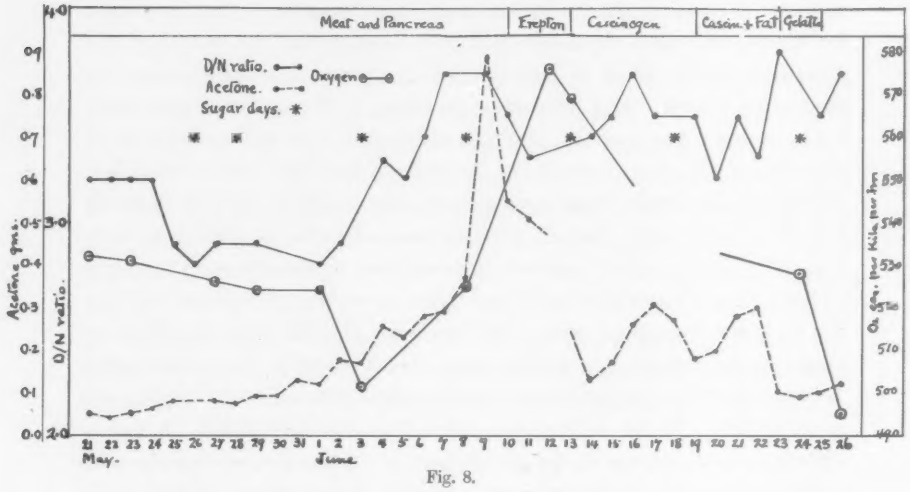


Fig. 8.

Dog VIII.

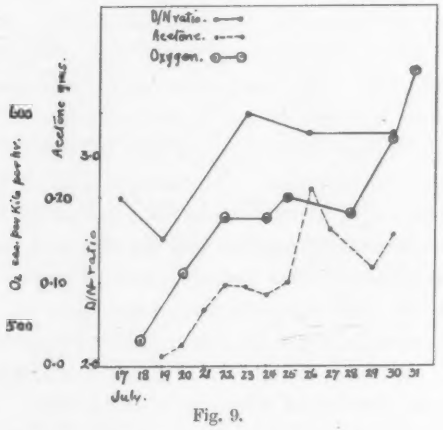


Fig. 9.

with the diabetics because of differences in size and weight. More recently, however, Benedict and Joslin in their report on cases of severe diabetes have

substantiated their former finding. Their later normal controls are strictly comparable in the matter of dimensions and the authors find the increase in diabetes more marked than previously stated. Taken as an isolated fact this increase in metabolism cannot be used as a deciding argument in favour of either the over-production or the non-utilisation theory. The fact that the total metabolism increases more and more during the course of the diabetes might be taken to indicate that this phenomenon is secondary to some other process. It is interesting, therefore, to find out what parallelism exists between the increase of total metabolism and other evidences of disturbance. In Figs. 8 and 9 the D : N ratio, the total acetone excretion, and the oxygen consumption in cc. per kilo. per hour of the diabetic Dogs VII and VIII, have been charted. The acetone excretion is subject to some fluctuation but a certain amount of parallelism exists between the course of the oxygen consumption and the acetone excretion. The three curves in Fig. 9 show a corresponding rise. In the case of Dog III when diabetic these three curves also show a parallel rise. There seems to be a relationship between the degree of disturbance of the protein metabolism and the oxygen consumption. As far as one can judge from the calculations of energy distribution an increase in fat metabolism or evidence of incomplete combustion of fat does not seem to bear a relation to the increase in total metabolism. Some interesting observations of Benedict and co-workers show that, in the normal human, an acidosis induced by a carbohydrate-free diet is accompanied by an increase in the total metabolism. It is apparently difficult to induce an acidosis in normal dogs and in some experiments with fat feeding (Dog VII, Table VIII) observations upon the acetone excretion and respiratory exchange did not show any marked variation. The part played by sugar usage in relation to the changes in total metabolism in diabetic dogs is an important consideration. The evidence as to the power to oxidise sugar, which is exhibited by animals during the course of the diabetes, consists in the level of the respiratory quotient and the degree of rise after sugar is given. From this evidence it has been seen that the degree of utilisation is sometimes less shortly after depancreatisation than later on. This is evident from the series of curves in Fig. 6 from the partially depancreatised Dog VII. In this case a limited degree of sugar usage occurred after three weeks. The course of the total metabolism does not seem to bear a close relation to the evidences of sugar usage. In the totally depancreatised animals the diminished utilisation of sugar is evident from the beginning of the diabetic period, while the oxygen consumption shows a steady increase throughout.

This comparison of the course of the two processes would seem to indicate that the disturbance of carbohydrate metabolism is the primary process and the gradual increase in total metabolism an evidence of a cumulative disturbance in protein and perhaps fat metabolism or an attempt to make up for the deficiency in the energy exchanges caused by the imperfect utilisation of sugar.

The increase in protein metabolism in diabetes has been noted by many investigators. The protein break-down is shown by the marked loss of nitrogen to the body especially in the earlier days of diabetes. In our experiments calculation of the energy distribution to protein after allowance has been made for sugar formation shows that the increase is 5 % to 10 % over the normal protein value in most cases. The actual protein break-down in the fasting condition has been shown to be enormously increased in pancreatic diabetes [Falta, Grote and Staehelin, 1907] and in phloridzin glycosuria [Reilly, Nolan and Lusk, 1898]. The protein destruction as evidenced by the nitrogen elimination shows an enormous increase, while the actual part taken by protein in the oxidations is only slightly increased. This process may be illustrated by the figures from two observations upon a dog in the normal and diabetic condition after the ingestion of meat.

(a) Dog III. Normal.

	CO_2	O_2	R.Q.
Feb. 12, 1914. Wt. 7·600	3768	4677 ⟶	0·806 (observed)
N in g. per hr. = 0·340 =	1662	2053	
	2106	2624 ⟶	0·802

in which the protein percentage is 44·0 %, carbohydrate 19 %, fat 37 %.

(b) Dog III. Diabetic 4 days. D : N ratio 3·8.

	CO_2	O_2	R.Q.
Feb. 20, 1914. Wt. 6·800	3132	4624 ⟶	0·680 (observed)

	CO_2	O_2	CO_2	O_2	R.Q.
N in g. per hr. = 0·595 =	2907	3592	1227	1912	
Sugar from protein = 2·26 g. =	1680	1680	1905	2712 ⟶	0·705
	1227	1912			

Protein 41·0 %. Carbohydrate 0·0 %. Fat 59·0 %.

Both observations are six hours after meat containing 4·0 g. nitrogen had been eaten. Hourly figures are given.

Comparison of these two observations will show that, while the protein percentage of the total oxygen distribution is practically the same in both cases, the nitrogen excretion, and therefore the protein destruction, is greatly increased.

In the protein break-down which occurs in diabetes the form in which the nitrogen is excreted might give some indication of the disturbance. Expressed in percentage of the total nitrogen the urea nitrogen tends to diminish and the ammonia to increase, and the increase in ammonia nitrogen corresponds generally with the increased excretion of acetone bodies. The balance thus maintained by the urea and ammonia nitrogen is an expression of the process by which the organism attempts to counteract the increasing acidosis, as has been generally recognised in cases of human diabetes. The destruction of tissue protein is shown also in the increase of the purine metabolism in diabetes. The allantoin excretion in one dog where the estimation in the presence of sugar is satisfactory formed up to one gram of the total nitrogen or about 10 %. The allantoin nitrogen in the normal animal under the same conditions of diet amounts to 0·5 g., or 3 % of the total nitrogen. The excretion of uric acid in the normal dog is very small and showed a definite increase in diabetes. The phosphate excretion in diabetes is slightly greater than the intake, but the phosphate balance shows such an inconsiderable loss that it cannot be taken as evidence of tissue destruction.

The protein destruction, as shown by the great loss of nitrogen to the body, starts soon after the removal of the pancreas, and the intake has to be greatly increased before a balance is reached. In this increase of the protein metabolism in the first few days of diabetes the amount of nitrogen excreted per hour is equal to that observed after a protein meal in the normal condition, but the respiratory exchanges do not show the increase in total metabolism which would occur were the protein fully oxidised. If, on the basis of the D : N ratio, we subtract the energy equivalent of the sugar formed from protein, the energy distribution to protein in the total metabolism is found to be somewhat increased. These facts indicate that the well recognised break-down of protein is closely associated with the power of the tissues to oxidise carbohydrate.

The question as to what significance can be attached to the steadily increasing excretion of acetone bodies in experimental diabetes is of interest. The possible sources of these intermediary products are the proteins or fats, and some indication has been sought for as to whether they originate mainly from alteration in the protein or fat metabolism. On a carbohydrate-free

diet normal dogs do not show any marked increase of acetone excretion. With feeding of fats to Dog VII no such increase was observed. As soon as pancreatic diabetes is established the acetone excretion increases steadily and shows a parallel course to the D : N ratio and the increase in total metabolism. If the acetone bodies had their source in incomplete fat combustion one would expect the respiratory quotient to fall in relation to the rise in acetone excretion. This, however, does not appear to be the case. The energy distribution calculations on days when the acetone excretion is highest do not show any marked indication of incomplete fat oxidation. The total acetone excretion in the diabetic dogs is not large and the production of a small amount might not influence the level of the respiratory quotient. The general course of the acetone excretion bears a closer relation to the protein metabolism. Variations in the total nitrogen are usually accompanied by parallel variations in the total acetone. In view of the fact that amino-acids, such as leucine and tyrosine, have been observed to produce acetoacetic acid, while many of the other amino-acids of protein produce sugar in diabetes, the influence of feeding different proteins and erepton was studied in Dog VII. In this dog the acetone excretion reached its highest level upon the standard diet of horse meat and pancreas. Upon erepton the acetone excretion was high and fell somewhat with caseinogen feeding. When gelatin was fed the acetone excretion fell considerably while the D : N ratio rose. We have therefore some indication that the acetone excretion depends on the character of the proteins fed. In the case of gelatin, which contains a larger proportion of sugar-producing amino-acids, the results are well marked. The most important source of the acetone bodies in the diabetic metabolism of dogs appears to be protein, and the increasing disturbance in the protein metabolism is indicated by the excretion of the acetone bodies as well as by the D : N ratio.

Utilisation of Sugar in Diabetes.

The generally low level of the respiratory quotient in diabetes is one of the chief arguments in favour of the non-utilisation theory. It might be argued, however, that in diabetes other processes which require much oxygen and yield less carbon dioxide obscure an oxidation of carbohydrate which is as effective as in the normal condition. The processes which would contribute to give very low respiratory quotients are the following:

(*a*) The formation of sugar from protein. Magnus Levy has calculated that the respiratory quotient yielded by protein after the full amount of

sugar formed is allowed for, is 0·615. In our calculations with a D : N ratio ranging from 2·7 to 3·8 the respiratory quotient of protein decreases from 0·715 to 0·640.

(*b*) The formation of acetone bodies from protein.

(*c*) The formation of acetone bodies from fat.

(*d*) The formation of sugar from fat.

We have attempted in our calculations to allow for the formation of sugar from protein. The formation of acetone bodies has not been taken into account in the calculations, because the small amounts excreted indicate that the part played by this process in the low respiratory quotient is a minor one. The respiratory quotient of the formation of sugar from fat is very low. The figures cannot be corrected for the formation of sugar from fat because there are no data to go upon. If this process occurred to any extent in diabetes the respiratory quotient should reach much lower values than those generally observed.

After making allowance for the first of the factors mentioned above, we still find very little evidence of sugar utilisation in the diabetic dogs, and the apparent utilisation when it occurs may be due to error in the use of the D : N ratio in the calculation.

The diminished rise or constant level of the respiratory quotient after giving glucose to diabetic dogs is also evidence of the inability of the tissues to oxidise glucose. The course of the respiratory quotient agrees with the percentage of sugar recovered from the urine. When a rise of the respiratory quotient does occur, after giving sugar to totally depancreatised animals it is in most cases small and within the limit of possible error. The disturbance of sugar utilisation is apparent shortly after depancreatisation and does not appear to increase with the severity of the diabetes. The giving of sugar later in the course of the diabetes resulted in a limited utilisation in the case of Dog VIII. The rise in the respiratory quotient after fructose (Table VI, protocol *o*) indicated a utilisation of 0·30 g. of sugar per hour during the observations. After glucose (protocol *p*) the utilisation was 0·25 g. per hour in the same dog three days later. In the partially depancreatised Dog VII the course of the sugar utilisation is similar, but the ability to oxidise sugar returned to a greater degree. In this latter case the return of the sugar usage can be explained by the pancreas tissue present, which evidently became more functional after a time had elapsed. An explanation of the apparent partial recovery in the power of using sugar in Dog VIII is harder to find. Remnants of pancreas tissue were carefully searched for at the

post-mortem without result. It may be that the tissues elaborate to some degree some other way of dealing with carbohydrate than that which exists when the pancreas is intact. Another possibility is that the increasing number of organisms in the intestinal tract and elsewhere account for the oxidation of sugar which occurs. The general condition of the animals has usually corresponded with the ability to use carbohydrate; thus Dog VIII after total depancreatisation was in much better condition than any of the other diabetic dogs. The results, therefore, indicate that removal of the pancreas in dogs causes a serious disturbance in the carbohydrate metabolism, in which the power of oxidising glucose is, for a time, completely lost and, while this may return to a small degree, it is never again observed to approach the normal in extent.

The recent work of Verzar [1914] on the question of sugar utilisation in diabetes has also given support to the non-utilisation theory. Verzar finds that, when sugar is injected into the circulation of diabetic animals, the normal rise of the respiratory quotient does not occur, but that this evidence of the loss of sugar utilisation is obtained only some days after the removal of the pancreas. In our experiments the effect of sugar giving was tried for the first time about the third or fourth day after depancreatisation, and only in the case of Dog III did we obtain evidence of a limited sugar utilisation two days after the operation. The results obtained from the injection of glucose and fructose therefore lead to the same conclusions as our findings in regard to the sugar utilisation in diabetes.

Summary.

1. In experimental diabetes an increase of the total metabolism occurs, amounting to 15 to 20 % on an average, and increasing with the severity of the condition.

2. The rise of the respiratory quotient which normally occurs after the giving of glucose or fructose is greatly diminished or is absent after the total removal of the pancreas, indicating that complete oxidation of sugar does not occur.

3. The increase in the excretion of acetone bodies parallels the increase in the D : N ratio and the total metabolism, suggesting that the phenomena are expressions of a similar disturbance of protein metabolism which is secondary to the impairment of sugar utilisation.

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Date	Diet (g. per diem)				Urine (g				
		N	Carbo-hydrate	Fat	Total N	Urea N	Allant. N	NH_3 N	Amino
Dog I									
Jan. 30*	.....	—	—	—	—	—	—	—	—
„ 31	—	0	0	0	8·29	6·86	—	0·60	0·03
Feb. 1	Meat	9·0	—	—	8·23	6·86	—	0·51	0·07
„ 2	„	5·0	—	—	8·15	6·63	—	0·45	0·08
„ 3	„	5·0	—	—	6·72	4·9	—	0·55	0·07
„ 4	—	0·0	15·0	—	1·85	1·26	—	0·15	0·05
„ 5	—	—	—	—	—	—	—	—	—
Dog III									
Feb. 10	Meat and Milk	6·1	5·0	3·0	4·06	3·47	0·22	0·12	0·02
„ 11	„ „	7·5	39·0	5·0	9·58	8·62	0·20	0·35	0·07
„ 12	Meat	7·5	0	0	8·18	7·33	0·28	0·27	0·05
„ 13	„	7·5	—	—	7·14	6·24	0·34	0·25	0·05
„ 14	„	8·8	—	—	9·55	8·46	0·22	0·31	0·04
„ 15	„	8·8	—	—	6·94	6·16	0·20	0·19	0·03
„ 16*	.....	0	0	0	3·19	2·83	—	0·06	0·02
„ 17	—	0	0	0	6·13	5·04	—	0·30	0·04
„ 18	Meat and Panc.	8·3	35·0	3·0	8·27	6·89	—	0·36	0·06
„ 19	„ „	8·3	25·0	3·0	7·90	6·72	—	0·38	0·04
„ 20	„ „	8·3	—	—	14·28	12·43	—	0·71	0·05
„ 21	„ „	7·4	20·0	—	10·21	8·74	—	0·47	0·07
„ 22	„ „	8·8	—	—	15·62	13·47	—	0·90	0·08
„ 23	—	—	—	—	—	—	—	—	—
Dog IV									
Mar. 2*	.....	—	—	—	—	—	—	—	—
„ 3	Meat and Panc.	0	0	0	5·10	—	—	—	—
„ 4	M. and P.	9·0	—	—	7·06	—	—	—	—
„ 5	„	9·0	—	—	8·36	—	—	—	—
„ 6	—	—	30·0	—	5·0	—	—	—	—
„ 7	—	—	—	—	—	—	—	—	—
Dog V									
Mar. 2*	.....	—	—	—	—	—	—	—	—
„ 3	M. and P.	0	0	0	5·88	—	—	—	—
„ 4	„	10·6	—	—	13·05	—	—	—	—
„ 5	„	5·0	—	—	10·92	—	—	—	—
„ 6	—	0	—	—	7·62	—	—	—	—
„ 7	—	0	—	—	2·49	—	—	—	—
„ 8	—	—	—	—	—	—	—	—	—
Dog VII									
Mar. 10	Caseinogen and Palmine	6·6	—	30·0	6·92	6·02	0·25	0·39	0·04
„ 11	„ „	4·0	—	35·0	3·64	2·97	0·31	0·25	0·01
„ 12	„ „	6·6	—	30·0	5·85	5·01	0·22	0·28	0·03
„ 13	„ „	6·6	—	30·0	6·38	5·52	0·17	0·40	0·01
„ 14	„ „	6·6	—	30·0	6·33	5·49	0	0·31	0
„ 15	„ „	6·6	—	40·0	6·33	5·32	0·22	0·49	0
„ 16	„ „	6·6	—	40·0	6·13	5·27	0·28	0·38	0·05
„ 17	Cas. and cod liver oil	6·6	—	40·0	6·52	5·68	0·20	0·41	0·03
„ 18	„ „	6·6	—	40·0	5·91	5·21	0·20	0·27	0·03
„ 19	„ „	6·6	—	40·0	5·96	5·26	0·20	0·29	0·01
„ 20	„ „	6·6	—	40·0	—	—	—	—	—
„ 21	„ „	6·6	—	40·0	—	—	—	—	—
„ 22	„ „	6·6	—	40·0	—	—	—	—	—
„ 23	Palmine	6·6	—	40·0	—	—	—	—	—
„ 24	„	6·6	—	40·0	—	—	—	—	—
„ 25	„	6·6	—	50·0	—	—	—	—	—
„ 26	„	6·6	—	50·0	—	—	—	—	—
„ 27	Butter	6·6	—	50·0	—	—	—	—	—
„ 28	„	6·6	—	50·0	—	—	—	—	—
May 12	Meat	10·0	—	—	9·8	8·65	0·20	0·50	0·01
„ 13	„	10·0	—	—	10·1	8·7	0·17	0·50	0·02
„ 14	„	10·0	20·0	—	—	—	—	—	—
„ 15	„	10·0	—	—	9·9	8·8	0·14	0·50	0·11
„ 16	„	10·0	—	—	10·2	8·8	0·34	0·50	0·03
„ 17	„	10·0	—	—	8·7	7·5	0·25	0·40	0·01
„ 18*	.....	0	0	0	—	—	—	—	—
„ 19	Milk	0·5	5·0	4·0	7·8	6·3	—	0·47	0·01
„ 20	„	1·0	10·0	8·0	7·8	6·7	—	0·37	0·02

TABLE VIII. *Totally Depancreatised Dogs I, III, IV, V, VII and VIII.*

Urine (g. per diem)							Faeces			% of Total Nitro			
N	Amino N	Creat. N	Uric Acid mgm.	P_2O_5	Sugar	Acetone	D/N	N	Fat	P_2O_5	Urea	Allant.	NH_3	Amino
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0·03	0·05	35+	1·00	26·0	—		3·1	—	—	—	82·8	3·0?	7·2	0·4
0·07	0·05	35+	1·17	24·7	—		3·0	—	—	—	83·5	—	6·2	0·9
0·08	0·06	35+	1·36	22·0	—		2·7	—	—	—	81·4	—	5·6	0·9
0·07	0·05	—	1·16	13·0	—		1·9	3·2	8·2	1·21	72·9	5·8?	8·1	1·0
0·05	0·02	—	0·38	6·0	—		3·2	1·6	3·3	0·50	68·2	—	8·2	2·1
—	—	—	—	—	—		—	—	—	—	—	—	—	—
0·02	0·06	0·0	0·61	—	—		—	—	—	—	85·5	5·5	2·9	0·4
0·07	0·09	0·0	1·14	—	0·009		—	—	—	—	90·1	2·0	3·6	0·7
0·05	0·07	0·0	0·76	—	0·011		—	—	—	—	89·7	3·4	3·3	0·6
0·05	0·07	0·0	0·72	—	0·009		—	0·952	1·50	0·47	87·4	4·7	3·5	0·6
0·04	0·08	0·0	1·14	—	0·011		—	—	—	—	88·6	2·3	3·3	0·4
0·03	0·06	0·0	0·81	—	0·023		—	0·43	2·30	0·17	88·7	2·8	2·7	0·5
0·02	0·05	19·0	0·75	1·4	0·007		—	—	—	—	88·6	6·1	1·9	0·7
0·04	0·06	27·0	1·23	18·0	0·012		2·7	—	—	—	82·2	9·6	4·8	0·7
0·06	0·05	45·5	1·75	57·5	0·031		6·9	—	—	—	83·2	1·1?	4·4	0·7
0·04	0·03	48·0	1·52	48·0	0·074		6·0	—	—	—	85·1	8·2	4·8	0·6
0·05	0·03	45·5	1·93	54·2	0·169		3·8	1·30	3·2	0·66	87·0	—	5·0	0·3
0·07	0·03	45·0	1·48	54·9	0·163		5·4	0·91	1·8	0·45	85·6	—	4·6	0·6
0·08	0·04	48·0	1·94	52·5	0·242		3·4	1·30	1·7	0·56	86·2	—	5·8	0·5
—	—	—	—	—	—		—	—	—	—	—	—	—	—
—	—	—	—	—	—		—	—	—	—	—	—	—	—
—	—	—	0·58	14·7	0·180		2·9	—	—	—	—	—	—	—
—	—	—	0·86	25·4	0·076		3·5	1·12	2·06	0·34	—	—	—	—
—	—	—	1·27	32·1	0·161		3·8	1·13	4·8	0·39	—	—	—	—
—	—	—	0·91	38·6	0·144		7·7	—	—	—	—	—	—	—
—	—	—	—	—	—		—	—	—	—	—	—	—	—
—	—	—	—	—	—		—	—	—	—	—	—	—	—
—	—	—	0·86	17·2	0·054		2·9	—	—	—	—	—	—	—
—	—	—	2·27	46·5	0·112		3·5	—	—	—	—	—	—	—
—	—	—	1·52	32·0	0·116		3·0	2·15	4·03	0·37	—	—	—	—
—	—	—	1·53	24·0	0·074		3·0	—	—	—	—	—	—	—
—	—	—	0·39	10·2	0·027		4·0	—	—	—	—	—	—	—
—	—	—	—	—	—		—	—	—	—	—	—	—	—
0·04	0·06	—	0·90	—	0·026		—	—	—	—	—	—	—	—
0·01	0·06	—	0·43	—	0·034		—	—	—	—	—	—	—	—
0·03	0·08	—	0·84	—	0·008		—	0·55	2·34	1·27	—	—	—	—
0·01	0·08	—	0·75	—	0·006		—	0·46	1·09	1·49	—	—	—	—
0	0·08	—	0·80	—	0·008		—	0·53	0·90	1·58	—	—	—	—
0	0·08	—	0·85	—	0·005		—	0·52	0·88	1·43	—	—	—	—
0·05	0·08	—	0·81	—	0·282?		—	—	—	—	—	—	—	—
0·03	0·07	—	0·85	—	0·038?		—	0·94	2·27	2·64	—	—	—	—
0·03	0·07	—	0·71	—	0·007		—	0·55	0·67	1·75	—	—	—	—
0·01	0·06	—	0·73	—	0·011		—	0·49	0·44	1·76	—	—	—	—
—	—	—	—	—	0·006		—	—	—	—	—	—	—	—
—	—	—	—	—	0·011		—	—	—	—	—	—	—	—
—	—	—	—	—	0·010		—	—	—	—	—	—	—	—
—	—	—	—	—	0·010		—	—	—	—	—	—	—	—
—	—	—	—	—	0·004		—	—	—	—	—	—	—	—
—	—	—	—	—	0·011		—	—	—	—	—	—	—	—
—	—	—	—	—	0·006		—	—	—	—	—	—	—	—
—	—	—	—	—	0·007		—	—	—	—	—	—	—	—
—	—	—	—	—	0·007		—	—	—	—	—	—	—	—
0·01	0·09	—	0·86	—	—		—	—	—	—	88·2	2·0	5·1	0·11
0·02	0·06	—	1·0	—	—		—	—	—	—	86·1	1·7	5·0	0·17
—	—	—	1·2	—	0·012		—	1·84	2·8	1·66	—	—	—	—
0·11	0·06	—	1·04	—	0·010		—	—	—	—	88·8	1·4	5·5	0·11
0·03	0·07	—	1·14	—	0·036		—	—	—	—	86·2	3·4	5·0	0·33
0·01	0·06	—	0·91	—	0·010		—	1·27	1·73	0·56	86·2	3·0	4·6	0·13
—	—	—	—	0	—		—	—	—	—	—	—	—	—
0·01	0·08	—	0·61	1·85	0·012		0·2	—	—	—	80·8	—	6·0	0·07
0·02	0·07	—	1·04	30·0	0·021		3·8	—	—	—	86·0	—	4·8	0·3

Nitrogen							
ino	Creatin.	Undet. N	Temp.	Pulse rate	Resp. rate	Weight	Notes
	—	—	—	—	—	7·0	Depancreatised.
4	0·7	5·9	37·6	—	17	6·4	Bright and active.
9	0·6	8·8	—	—	—	6·350	
9	0·6	—	—	—	—	6·100	Depressed and weak.
0	0·7	11·5	—	—	—	5·800	Very depressed and weak; coughing.
1	1·0	—	—	—	—	5·600	Very weak; coughing; vomiting; diarrhoea; will not eat.
	—	—	35·0	100	—	5·200	Extreme weakness; died under anaesthetic.
4	1·5	4·2	—	—	—	7·6	
7	0·9	2·7	—	—	—	—	
5	0·9	2·1	—	—	—	—	
5	0·9	2·9	—	—	—	—	
4	0·8	4·6	—	—	—	—	
5	0·9	4·4	—	—	—	7·500	
	1·6	1·6	—	—	—	—	Depancreatised.
	0·9	1·8	—	—	—	7·200	Bright and active.
	0·6	10·0	—	—	—	—	
	0·4	0·9	—	—	—	—	Bright; marked appetite.
	0·2	6·5	—	—	—	—	
	0·3	8·5	—	—	—	6·750	In good condition; wound closing and clean.
	0·3	7·2	—	—	—	6·500	
	—	—	—	—	—	—	Died under anaesthetic.
	—	—	—	—	—	—	Depancreatised; moving about 4 hours after operation.
	—	—	—	—	—	8·000	
	—	—	—	—	—	7·600	Bright and active; eats readily.
	—	—	—	—	20–42	7·600	Somewhat depressed; refused part ration.
	—	—	—	—	—	7·300	Vomiting; some suppuration about wound; Conjunctivitis.
	—	—	—	—	—	—	Vomiting and diarrhoea; very weak; died night of Mar. 7.
	—	—	—	—	—	—	Depancreatised.
	—	—	—	—	—	6·400	Normal weight.
	—	—	—	—	—	5·850	Bright; appetite good.
	—	—	—	110	15	7·650	Depressed and weak; appetite bad ; vomiting.
	—	—	—	—	—	—	Vomiting; diarrhoea; refuses food.
	—	—	—	—	—	4·950	Bad condition; conjunctivitis; vomiting; diarrhoea
	—	—	—	—	—	—	Dog killed under chloroform.
	—	—	—	—	—	9·000	
	—	—	—	—	—	—	Refused part of ration.
	—	—	—	—	—	—	
	—	—	—	—	—	8·900	
	—	—	—	—	—	—	
	—	—	—	—	—	—	
	—	—	—	—	—	8·800	? Rise not due to acetone.
	—	—	—	—	—	—	" " " "
	—	—	—	—	—	8·750	
	—	—	—	—	—	—	
	—	—	—	—	—	8·750	
	—	—	—	—	—	—	
	—	—	—	—	—	—	
	—	—	—	—	—	—	
	—	—	—	—	—	—	
	—	—	—	—	—	8·750	
	—	—	—	—	—	—	
	—	—	—	—	—	—	
	—	—	—	—	—	—	
1	—	—	—	—	—	—	
7	—	—	37·8	80	15	9·150	
	—	—	—	—	—	—	
1	—	—	—	—	—	—	
3	—	—	—	—	—	—	
	—	—	—	—	—	—	
	—	—	—	—	—	—	Partial depancreatisation; graft brought up under skin of abdomen.
7	—	—	—	—	—	—	
	—	—	—	—	—	8·650	
						8·500	Large swelling about abdominal wound.

„	19	Milk	0·5	5·0	4·0	7·8	6·3	—	0·47	0·01	0·08
„	20	„	1·0	10·0	8·0	7·8	6·7	—	0·37	0·02	0·07
„	21	M. and P.	6·0	—	—	6·4	5·4	—	0·45	0	0·05
„	22	„	8·5	—	—	9·6	8·2	—	0·63	0·01	0·06
„	23	„	8·5	—	—	10·3	8·9	—	0·59	0·01	0·06
„	24	„	8·5	—	—	10·25	8·7	—	0·67	0·01	0·07
„	25	„	8·5	—	—	11·87	10·2	—	0·75	0·01	0·07
„	26	„	8·5	20·0	—	10·5	8·9	—	0·72	0·01	0·04
„	27	„	8·5	—	—	9·8	8·9	—	0·56	0·04	0·06
„	28	„	7·5	20·0	—	8·0	6·7	—	0·45	0·05	0·06
„	29	„	7·5	—	—	8·2	6·7	—	0·59	0·01	0·06
„	30	„	7·5	—	—	7·56	6·2	—	0·53	0·01	0·06
„	31	„	7·5	—	—	9·18	7·5	—	0·56	0·01	0·06
June	1	„	7·5	—	—	7·28	6·0	—	0·53	0·01	0·05
„	2	„	7·5	—	—	7·7	6·2	—	0·62	0·01	0·05
„	3	„	7·5	20·0	—	7·68	—	—	—	—	—
„	4	„	9·5	—	—	10·6	9·1	—	0·77	0·02	0·05
„	5	„	9·5	—	—	10·0	8·4	—	0·73	0·01	0·05
„	6	„	9·5	—	—	10·5	9·3	—	0·29	0·01	0·05
„	7	„	9·5	—	—	10·7	9·1	—	0·47	—	0·05
„	8	„	10·0	20·0	—	11·2	—	—	—	—	—
„	9	„	10·0	—	—	11·9	10·13	—	0·70	0·02	—
„	10	M., P. and Erept.	10·0	—	—	11·2	9·0	—	0·90	0·03	—
„	11	„ „	10·0	—	—	12·0	10·05	—	0·88	0·03	—
„	12	„ „	10·0	—	—	—	—	—	—	—	—
„	13	Cas. and Pan.	9·6	20·0	—	9·6	—	—	—	—	—
„	14	„	9·6	—	—	11·0	9·4	—	0·80	0·05	—
„	15	„	12·9	—	—	13·0	11·1	—	0·94	0·17	—
„	16	„	12·9	—	—	13·9	11·6	—	1·02	0·39	—
„	17	„	12·9	—	—	13·1	10·7	—	1·15	0·26	—
„	18	„	12·9	—	—	11·3	—	—	—	—	—
„	19	Cas., Pan. and Palmine	12·9	—	3·0	12·5	10·8	—	0·80	0·14	—
„	20	„ „	12·9	—	10·0	13·3	11·3	—	1·02	0·14	—
„	21	„ „	12·9	—	10·0	12·7	10·64	—	1·00	0·32	—
„	22	„ „	12·9	—	10·0	12·3	10·2	—	1·12	0·21	—
„	23	Gelatin	12·9	—	—	12·9	12·0	—	0·70	0·00	—
„	24	„	12·9	20·0	—	13·2	—	—	—	—	—
„	25	Caseinogen and cod liver oil	12·9	—	10·0	12·3	10·3	—	1·00	0·01	—
„	26	„ „ „	12·9	—	10·0	10·4	8·68	—	0·80	0·02	—
„	27	—	—	—	—	—	—	—	—	—	—

Dog VIII

May	14	—	—	—	—	—	—	—	—	—	—
„	15	—	—	—	—	—	—	—	—	—	—
„	16	—	—	—	—	—	—	—	—	—	—
„	17	—	—	—	—	8·34	—	—	—	—	—
„	18*		—	—	—	—	—	—	—	—	—
„	19	Milk	0·5	5·0	3·0	6·75	—	—	—	—	—
„	20	„	0·85	5·0	3·0	9·5	—	—	—	—	—
„	21	Meat and Panc.	4·3	—	—	7·28	—	—	—	—	—
„	22	„ „	5·5	—	—	5·88	—	—	—	—	—
„	23	„ „	5·5	—	—	5·85	—	—	—	—	—
„	24	„ „	5·5	—	—	8·8	—	—	—	—	—
„	25	„ „	5·5	20·0	—	5·57	—	—	—	—	—
„	26	„ „	5·5	—	—	4·46	—	—	—	—	—
„	27	„ „	5·5	10·0	—	4·52	—	—	—	—	—
„	28	„ „	5·5	—	—	5·0	—	—	—	—	—
„	29	„ „	5·5	20·0	—	3·9	—	—	—	—	—
„	30	„ „	5·5	—	—	4·7	—	—	—	—	—
„	31	„ „	5·5	—	—	5·0	—	—	—	—	—
June	1	„ „	5·5	—	—	4·3	—	—	—	—	—
„	2	„ „	5·5	—	—	3·5	—	—	—	—	—
„	3	P.b. and Meat	6·5	30·0	—	4·9	—	—	—	—	—
„	4	„ „	6·5	30·0	—	5·0	—	—	—	—	—
„	5	Meat and Panc.	6·9	20·0	—	5·9	—	—	—	—	—
„	6	„ „	6·9	—	—	3·9	—	—	—	—	—
„	7	„ „	6·9	—	—	6·2	—	—	—	—	—
„	8	„ „	6·9	—	—	5·8	—	—	—	—	—
„	9	„ „	6·9	20·0	—	6·2	—	—	—	—	—
„	10	„ „	—	—	—	—	—	—	—	—	—
„	11	„ „	—	—	—	—	—	—	—	—	—
„	16	Meat, Panc. and Glucose	6·5	30·0	—	5·5	—	—	—	—	—
„	17	„ „	6·5	30·0	—	4·2	—	—	—	—	—
„	18	„ „	6·5	50·0	—	4·3	—	—	—	—	—
„	19	„ „	6·5	50·0	—	5·3	—	—	—	—	—
„	20	Bread and Meat	6·5	100·0	—	4·0	—	—	—	—	—
„	21	„ „	6·5	100·0	—	5·3	—	—	—	—	—
„	29	Meat and P.b.	7·0	60·0	—	3·9	3·25	—	0·17	0	—
„	30	„	7·0	60·0	—	3·9	3·3	—	0·18	0·01	—
July	1	Meat, P.b. and Glucose	7·0	80·0	—	4·44	3·74	—	0·17	0·02	—

0·08	—	0·61	1·85	0·012	0·2	—	—	—	80·8	—	6·0	0·07	—	—	—	
0·07	—	1·04	30·0	0·021	3·8	—	—	—	86·0	—	4·8	0·3	—	—	—	
0·05	—	—	27·0	0·047	4·2	—	—	—	84·4	—	7·0	—	—	—	—	
0·06	—	1·43	22·5	0·039	2·3	—	—	—	85·1	—	6·5	0·06	—	—	—	
0·06	—	0·81	33·0	0·050	3·2	2·8	13·5	1·2	86·7	—	5·8	0·01	—	—	—	
0·07	—	1·04	33·0	0·06	3·2	—	—	—	84·8	—	6·5	0·11	—	—	—	
0·07	—	1·20	35·0	0·083	2·9	—	—	—	85·9	—	6·4	0·05	—	—	—	
0·04	—	1·52	50·3	0·076	4·7	0·85	4·9	0·35	84·8	—	6·8	0·1	—	—	—	
0·06	—	1·44	28·0	0·08	2·8	—	—	—	90·8	—	5·7	0·4	—	—	—	
0·06	—	1·34	43·0	0·072	5·4	—	—	—	83·7	—	5·6	0·67	—	—	—	
0·06	—	1·32	24·0	0·089	2·9	—	—	—	81·7	—	7·2	0·06	—	—	—	
0·06	—	1·42	20·0	0·09	2·6	—	—	—	82·0	—	7·0	0·07	—	—	—	
0·06	—	1·57	23·0	0·127	2·5	—	—	—	81·3	—	5·0	0·12	—	—	—	
0·05	—	1·22	21·0	0·120	2·8	2·26	10·5	0·68	82·4	—	7·3	0·15	—	—	—	
0·05	—	1·36	22·0	0·182	2·9	—	—	—	80·8	—	8·1	0·07	—	—	—	
—	—	1·24	39·8	0·174	5·1	—	—	—	—	—	—	—	—	—	—	
0·05	—	1·53	35·7	0·258	3·3	—	—	—	85·8	—	7·2	0·16	0·5	—	—	
0·05	—	1·66	32·0	0·226	3·2	—	—	—	84·0	—	7·3	0·11	0·5	—	—	
0·05	—	1·85	35·7	0·278	3·4	1·89	4·32	0·675	88·6	—	2·8	0·1	0·49	—	—	
0·05	—	1·72	40·0	0·286	3·7	1·93	3·64	0·57	85·0	—	4·4	—	0·44	—	—	
—	—	1·75	55·0	0·370	5·0	—	—	—	—	—	—	—	—	—	—	
—	—	1·99	44·0	0·896	3·7	—	—	—	85·7	—	6·0	—	—	—	37·0	
—	—	1·94	39·8	0·548	3·5	—	—	—	80·5	—	8·0	—	—	—	—	
—	—	1·91	40·0	0·512	3·3	1·83	2·9	0·74	83·7	—	7·0	—	—	—	—	
—	—	—	—	—	—	0·96	1·85	2·28	—	—	—	—	—	—	—	
—	—	—	48·7	—	5·0	—	—	—	—	—	—	—	—	—	—	
—	—	1·58	37·6	0·136	3·4	—	—	—	85·5	—	7·0	—	—	—	37·0	
—	—	1·69	46·7	0·172	3·5	—	—	—	85·2	—	7·0	—	—	—	—	
—	—	1·80	49·0	0·257	3·7	1·60	0·79	1·37	83·5	—	7·0	—	—	—	37·2	
—	—	1·85	46·0	0·310	3·5	1·30	2·10	3·0	81·7	—	8·0	—	—	—	—	
—	—	—	43·5	0·276	3·8	—	—	—	—	—	—	—	—	—	—	
—	—	1·38	43·8	0·188	3·5	1·01	1·91	2·64	86·4	—	6·0	—	—	—	—	
—	—	1·50	42·8	0·204	3·2	1·4	2·98	3·48	83·8	—	7·0	—	—	—	—	
—	—	1·44	44·6	0·288	3·5	—	—	—	83·8	—	7·0	—	—	—	—	
—	—	1·36	41·3	0·302	3·3	1·78	4·66	3·62	83·0	—	9·0	—	—	—	—	
—	—	0·77	49·0	0·108	3·8	0·36	1·28	1·26	97·0	—	5·0	—	—	—	—	
—	—	0·90	59·4	0·092	4·5	—	—	—	—	—	—	—	—	—	—	
—	—	1·55	43·4	0·112	3·5	—	—	—	83·0	—	8·0	—	—	—	—	
—	—	—	39·0	0·120	3·7	0·98	6·3	2·6	83·0	—	7·7	—	—	—	36·2	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	0·004	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	0·002	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	0·002	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	0·002	—	1·24	2·04	0·536	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	0·009	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	2·25	0·009	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	5·0	0·009	—	0·73	2·03	0·32	—	—	—	—	—	—	—	
—	—	—	6·0	0·008	—	2·82	5·15	0·63	—	—	—	—	—	—	—	
—	—	—	3·0	0·007	—	—	—	—	—	—	—	—	—	—	—	
—	—	0·68	5·1	0·006	—	1·2	1·86	0·23	—	—	—	—	—	—	—	
—	—	0·77	11·0	0·004	—	—	—	—	—	—	—	—	—	—	—	
—	—	0·81	1·69	0·032	—	2·42	5·94	0·70	—	—	—	—	—	—	—	
—	—	0·80	3·1	0·004	—	3·08	4·4	0·73	—	—	—	—	—	—	—	
—	—	0·75	0·0	0·014	—	0·68	1·7	0·28	—	—	—	—	—	—	—	
—	—	0·89	5·9	0·008	—	—	—	—	—	—	—	—	—	—	—	
—	—	0·89	0	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	0·95	0	—	—	0·64	1·3	0·30	—	—	—	—	—	—	—	
—	—	0·87	0	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	0·63	tr	—	—	0·45	0·62	0·22	—	—	—	—	—	—	—	
—	—	0·98	0	—	—	0·83	1·5	0·52	—	—	—	—	—	—	—	
—	—	0·84	0·35	—	—	0·74	1·24	0·74	—	—	—	—	—	—	—	
—	—	0·83	0	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	0·56	0	—	—	1·15	1·41	0·76	—	—	—	—	—	—	—	
—	—	1·01	0	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	1·01	0	—	—	0·92	1·43	0·40	—	—	—	—	—	—	—	
—	—	1·01	0	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	1·12	0	—	—	1·58	3·9	0·65	—	—	—	—	—	—	—	
—	—	—	—	—	—	1·13	1·4	0·52	—	—	—	—	—	—	—	
—	—	0·81	tr	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	0·75	tr	—	—	0·61	0·82	0·30	—	—	—	—	—	—	—	
—	—	0·87	1·5	—	—	1·02	1·5	0·59	—	—	—	—	—	—	—	
—	—	0·76	tr	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	0·59	tr	—	—	0·89	1·34	0·44	—	—	—	—	—	—	—	
—	—	0·58	tr	—	—	1·47	1·8	0·62	—	—	—	—	—	—	—	
—	—	—	—	—	—	—	—	—	83·3	—	4·0	—	—	—	—	
—	—	—	0	—	—	1·5	1·42	1·15	82·3	—	4·5	—	—	—	—	
—	—	—	0	—	—	0·91	0·89	0·50	84·3	—	4·0	—	—	—	—	
—	—	—	0	—	—	1·78	2·03	1·02	84·1	—	3·8	—	—	—	37	

							abdomen.
07	—	—	—	—	—	—	
3	—	—	—	—	—	8·650	
—	—	—	—	—	—	8·500	Large swelling about abdominal wound.
06	—	—	—	—	—	—	
01	—	—	—	—	—	8·100	Considerable amount of clear yellowish fluid discharged from opening in skin incision.
11	—	—	—	—	—	—	
05	—	—	—	—	—	—	Wound dressed twice daily.
1	—	—	—	—	—	—	
4	—	—	—	—	—	7·100	Dog active and bright; ravenous appetite.
57	—	—	—	—	—	7·100	
06	—	—	—	—	—	7·050	Clear fluid discharge from situation of graft, fluid digests fibrin in 20 hours. Loewi's test negative.
07	—	—	—	—	—	—	
12	—	—	—	—	—	—	
15	—	—	—	—	—	6·900	
07	—	—	—	—	—	—	
—	—	—	—	—	—	6·750	Dog not so active.
16	0·5	—	—	—	—	—	
11	0·5	—	—	—	—	—	
1	0·49	—	—	—	—	—	Wound completely closed and clean; sod. bicar. 10 g.
—	0·44	—	—	—	—	—	Sod. bicarb. 10 g. Intake 24·6 Output 23·67 urine, 3·475 faeces, 27·14
—	—	—	—	—	—	6·500	Dog very quiet.
—	—	—	37·0	50	13	—	Sod. bicarb. 10 g.
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	Rectal injection 10 g. sod. bicarb. in water.
—	—	—	—	—	—	6·200	" " " " "
—	—	—	—	34	10	—	
—	—	—	37·0	30	10	6·000	Dog very drowsy and weak.
—	—	—	—	—	—	—	
—	—	—	37·2	34	12	5·750	Seems better, less depressed.
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	Intravenous 30 cc. 5 % glucose.
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	Enema.
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	Enema.
—	—	—	—	—	—	—	Dog very weak and quiet.
—	—	—	—	—	—	5·200	Blood sugar estimations: before 0·25 %; after 0·40 % gluc.
—	—	—	—	—	—	—	Loewi's test negative.
—	—	—	36·2	32	10	5·000	Very weak and depressed.
—	—	—	—	—	—	—	Killed by chloroform.
—	—	—	—	—	—	—	
—	—	—	—	—	—	8·250	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	8·000	Operation I; partly depancreatised.
—	—	—	—	—	—	—	
—	—	—	—	—	—	7·750	Very active and hungry.
—	—	—	—	—	—	7·550	
—	—	—	—	—	—	—	
—	—	—	—	—	—	7·400	Abdominal skin incision slightly open
—	—	—	—	—	—	—	
—	—	—	—	—	—	7·200	
—	—	—	—	—	—	—	
—	—	—	—	—	—	7·000	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	Very well; abdominal skin incision healed.
—	—	—	—	—	—	—	
—	—	—	—	—	—	6·750	P_2O_5 intake 11·0; P_2O_5 output 10·5.
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	6·750	
—	—	—	—	—	—	—	Removed from metabolism cage.
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	Removed from cage.
—	—	—	—	—	—	6·500	
—	—	—	—	—	—	—	
—	—	—	—	—	—	6·500	

„	17	„	12·9	—	—	13·1	10·7	—	1·15	0·26	—
„	18	„	12·9	—	—	11·3	—	—	—	—	—
„	19	Cas., Pan. and Palmine	12·9	—	3·0	12·5	10·8	—	0·80	0·14	—
„	20	„ „ „	12·9	—	10·0	13·3	11·3	—	1·02	0·14	—
„	21	„ „ „	12·9	—	10·0	12·7	10·64	—	1·00	0·32	—
„	22	„ „ „	12·9	—	10·0	12·3	10·2	—	1·12	0·21	—
„	23	Gelatin	12·9	—	—	12·9	12·0	—	0·70	0·00	—
„	24	„	12·9	20·0	—	13·2	—	—	—	—	—
„	25	Caseinogen and cod liver oil ..	12·9	—	10·0	12·3	10·3	—	1·00	0·01	—
„	26	„ „ „ ..	12·9	—	10·0	10·4	8·68	—	0·80	0·02	—
„	27	— ..	—	—	—	—	—	—	—	—	—
Dog VIII											
May	14	— ..	—	—	—	—	—	—	—	—	—
„	15	— ..	—	—	—	—	—	—	—	—	—
„	16	— ..	—	—	—	—	—	—	—	—	—
„	17	— ..	—	—	—	8·34	—	—	—	—	—
„	18*		—	—	—	—	—	—	—	—	—
„	19	Milk	0·5	5·0	3·0	6·75	—	—	—	—	—
„	20	„	0·85	5·0	3·0	9·5	—	—	—	—	—
„	21	Meat and Panc.	4·3	—	—	7·28	—	—	—	—	—
„	22	„ „	5·5	—	—	5·88	—	—	—	—	—
„	23	„ „	5·5	—	—	5·85	—	—	—	—	—
„	24	„ „	5·5	—	—	8·8	—	—	—	—	—
„	25	„ „	5·5	20·0	—	5·57	—	—	—	—	—
„	26	„ „	5·5	—	—	4·46	—	—	—	—	—
„	27	„ „	5·5	10·0	—	4·52	—	—	—	—	—
„	28	„ „	5·5	—	—	5·0	—	—	—	—	—
„	29	„ „	5·5	20·0	—	3·9	—	—	—	—	—
„	30	„ „	5·5	—	—	4·7	—	—	—	—	—
„	31	„ „	5·5	—	—	5·0	—	—	—	—	—
June	1	„ „	5·5	—	—	4·3	—	—	—	—	—
„	2	„ „	5·5	—	—	3·5	—	—	—	—	—
„	3	P.b. and Meat	6·5	30·0	—	4·9	—	—	—	—	—
„	4	„ „	6·5	30·0	—	5·0	—	—	—	—	—
„	5	Meat and Panc.	6·9	20·0	—	5·9	—	—	—	—	—
„	6	„ „	6·9	—	—	3·9	—	—	—	—	—
„	7	„ „	6·9	—	—	6·2	—	—	—	—	—
„	8	„ „	6·9	—	—	5·8	—	—	—	—	—
„	9	„ „	6·9	20·0	—	6·2	—	—	—	—	—
„	10	„ „	—	—	—	—	—	—	—	—	—
„	11	„ „	—	—	—	—	—	—	—	—	—
„	16	Meat, Panc. and Glucose	6·5	30·0	—	5·5	—	—	—	—	—
„	17	„ „	6·5	30·0	—	4·2	—	—	—	—	—
„	18	„ „	6·5	50·0	—	4·3	—	—	—	—	—
„	19	„ „	6·5	50·0	—	5·3	—	—	—	—	—
„	20	Bread and Meat	6·5	100·0	—	4·0	—	—	—	—	—
„	21	„ „	6·5	100·0	—	5·3	—	—	—	—	—
„	29	Meat and P.b.	7·0	60·0	—	3·9	3·25	—	0·17	0	—
„	30	„	7·0	60·0	—	3·9	3·3	—	0·18	0·01	—
July	1	Meat, P.b. and Glucose	7·0	80·0	—	4·44	3·74	—	0·17	0·02	—
„	2	Meat, P.b. and Thyroid 1·0 ..	7·0	60·0	—	4·2	3·56	—	0·15	0·03	—
„	3	„ „ „ ..	7·0	60·0	—	4·5	4·0	—	0·19	0·03	—
„	4	Meat, P.b., Thy. 1·0 and Glucose	7·0	80·0	—	3·95	3·2	—	0·17	0·01	—
„	5	Meat, P.b. and Thyroid 1·5 ..	7·0	60·0	—	4·87	3·2	—	0·11	0·06	—
„	6	„ „ „ ..	7·0	60·0	—	5·3	4·5	—	0·17	0·03	—
„	7	„ „ 2·0 ..	7·0	60·0	—	3·9	3·3	—	0·112	0·02	—
„	8	„ „ 3·0 ..	7·0	60·0	—	4·7	3·86	—	0·196	0·04	—
„	9	„ „ Gluc. 3·0 ..	7·0	80·0	—	5·2	4·4	—	0·25	0·04	—
„	10	„ „ Thyroid 3·0 ..	7·0	60·0	—	5·0	4·1	—	0·30	0·03	—
„	11	Meat and Panc.	11·0	—	—	7·46	6·35	—	0·50	0·01	—
„	12	„ „	11·0	—	—	7·8	6·8	—	0·40	0·01	—
„	13	„ „	11·0	—	—	8·6	7·4	—	0·25	0·06	—
„	14	Meat, Panc. and Gluc.	11·0	20·0	—	8·96	7·8	0·25	0·34	0·03	—
„	15	Meat and Panc.	11·0	—	—	9·7	8·5	—	0·35	0·03	—
„	16	Meat, Panc. and Gluc.	11·0	20·0	—	9·3	8·2	—	0·40	0·01	—
„	17*		0	0	0	9·8	8·2	0·70	0·40	0·04	—
„	18	—	0	0	0	3·64	3·0	0·67	0·25	0	—
„	19	Meat and Panc.	6·0	—	—	8·71	7·5	0·56	0·42	0·02	—
„	20	Meat, Panc. and Gluc.	6·0	20·0	—	7·4	6·4	0·64	1·5	0·05	—
„	21	Meat and Panc.	11·8	—	—	9·86	8·9	1·16	0·67	0·02	—
„	22	Meat, Panc. and Gluc.	11·8	20·0	—	11·2	9·46	0·73	0·60	0·04	—
„	23	Meat and Panc.	11·8	—	—	11·9	9·7	1·03	0·81	0·05	—
„	24	Meat, Panc. and Gluc.	11·8	20·0	—	10·9	9·4	0·812	0·61	0·05	—
„	25	Meat and Panc.	11·8	—	—	10·1	8·15	1·01	0·67	0·03	—
„	26	„ „	11·8	—	—	11·2	9·2	1·03	0·81	0·05	—
„	27	„ „	10·0	—	—	9·5	—	—	—	—	—
„	28	Meat, Panc. and Fruct.	10·0	20·0	—	9·3	—	—	—	—	—
„	29	Meat, Panc. and Gluc.	11·8	20·0	—	11·5	—	—	—	—	—
„	30	Meat and Panc.	11·8	—	—	12·7	—	—	—	—	—
„	31	—	0	20·0	—	0·54	—	—	—	—	—

* Operation performed; see notes.

—	—	1·85	46·0	0·310	3·5	1·30	2·10	2·6	81·7	—	8·0	—	—	—	—
—	—	—	43·5	0·276	3·8	—	—	—	—	—	—	—	—	—	—
—	—	1·38	43·8	0·188	3·5	1·01	1·91	2·64	86·4	—	6·0	—	—	—	—
—	—	1·50	42·8	0·204	3·2	1·4	2·98	3·48	83·8	—	7·0	—	—	—	—
—	—	1·44	44·6	0·288	3·5	—	—	—	83·8	—	7·0	—	—	—	—
—	—	1·36	41·3	0·302	3·3	1·78	4·66	3·62	83·0	—	9·0	—	—	—	—
—	—	0·77	49·0	0·108	3·8	0·36	1·28	1·26	97·0	—	5·0	—	—	—	—
—	—	0·90	59·4	0·092	4·5	—	—	—	—	—	—	—	—	—	—
—	—	1·55	43·4	0·112	3·5	—	—	—	83·0	—	8·0	—	—	—	—
—	—	—	39·0	0·120	3·7	0·98	6·3	2·6	83·0	—	7·7	—	—	—	36·2
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	0·004	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	0·002	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	0·002	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	0·002	—	1·24	2·04	0·536	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	0·009	—	—	—	—	—	—	—	—	—	—	—
—	—	—	2·25	0·009	—	—	—	—	—	—	—	—	—	—	—
—	—	—	5·0	0·009	—	0·73	2·03	0·32	—	—	—	—	—	—	—
—	—	—	6·0	0·008	—	2·82	5·15	0·63	—	—	—	—	—	—	—
—	—	—	3·0	0·007	—	—	—	—	—	—	—	—	—	—	—
—	—	0·68	5·1	0·006	—	1·2	1·86	0·23	—	—	—	—	—	—	—
—	—	0·77	11·0	0·004	—	—	—	—	—	—	—	—	—	—	—
—	—	0·81	1·69	0·032	—	2·42	5·94	0·70	—	—	—	—	—	—	—
—	—	0·80	3·1	0·004	—	3·08	4·4	0·73	—	—	—	—	—	—	—
—	—	0·75	0·0	0·014	—	0·68	1·7	0·28	—	—	—	—	—	—	—
—	—	0·89	5·9	0·008	—	—	—	—	—	—	—	—	—	—	—
—	—	0·89	0	—	—	—	—	—	—	—	—	—	—	—	—
—	—	0·95	0	—	—	0·64	1·3	0·30	—	—	—	—	—	—	—
—	—	0·87	0	—	—	—	—	—	—	—	—	—	—	—	—
—	—	0·63	tr	—	—	0·45	0·62	0·22	—	—	—	—	—	—	—
—	—	0·98	0	—	—	0·83	1·5	0·52	—	—	—	—	—	—	—
—	—	0·84	0·35	—	—	0·74	1·24	0·74	—	—	—	—	—	—	—
—	—	0·83	0	—	—	—	—	—	—	—	—	—	—	—	—
—	—	0·56	0	—	—	1·15	1·41	0·76	—	—	—	—	—	—	—
—	—	1·01	0	—	—	—	—	—	—	—	—	—	—	—	—
—	—	1·01	0	—	—	0·92	1·43	0·40	—	—	—	—	—	—	—
—	—	1·01	0	—	—	—	—	—	—	—	—	—	—	—	—
—	—	1·12	0	—	—	1·58	3·9	0·65	—	—	—	—	—	—	—
—	—	—	—	—	—	1·13	1·4	0·52	—	—	—	—	—	—	—
—	—	0·81	tr	—	—	—	—	—	—	—	—	—	—	—	—
—	—	0·75	tr	—	—	0·61	0·82	0·30	—	—	—	—	—	—	—
—	—	0·87	1·5	—	—	1·02	1·5	0·59	—	—	—	—	—	—	—
—	—	0·76	tr	—	—	—	—	—	—	—	—	—	—	—	—
—	—	0·59	tr	—	—	0·89	1·34	0·44	—	—	—	—	—	—	—
—	—	0·58	tr	—	—	1·47	1·8	0·62	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	83·3	—	4·0	—	—	—	—
—	—	—	0	—	—	1·5	1·42	1·15	82·3	—	4·5	—	—	—	—
—	—	—	0	—	—	0·91	0·89	0·50	84·3	—	4·0	—	—	—	—
—	—	—	0	—	—	1·78	2·03	1·02	84·1	—	3·8	—	—	—	37·8
—	—	—	0	—	—	1·14	0·83	0·62	89·4	—	4·3	—	—	—	—
—	—	—	2·1	—	—	2·05	1·76	1·26	84·4	—	4·3	—	—	—	—
—	—	—	tr	—	—	2·2	1·5	1·38	81·1	—	2·5	—	—	—	—
—	—	—	tr	—	—	1·1	1·22	0·50	85·6	—	3·2	—	—	—	—
—	—	—	tr	—	—	1·3	1·7	0·63	83·6	—	3·0	—	—	—	—
—	—	—	tr	—	—	2·57	2·74	1·78	82·1	—	4·0	—	—	—	39·2
—	—	—	4·3	—	—	2·14	2·4	1·03	84·4	—	5·0	—	—	—	—
—	—	—	tr	—	—	1·73	2·02	1·04	82·0	—	6·0	—	—	—	—
—	—	—	0	—	—	1·34	2·9	1·4	85·1	—	6·5	—	—	—	—
—	—	—	0	—	—	—	—	—	87·1	—	5·0	—	—	—	—
—	—	—	0	—	—	1·54	3·77	1·23	87·9	—	3·0	—	—	—	—
—	—	—	4·8	—	—	1·28	2·67	0·87	87·2	2·7	3·0	—	—	—	—
—	—	—	0	—	—	—	—	—	87·4	—	3·5	—	—	—	—
—	—	—	3·3	—	—	1·40	2·35	0·81	88·3	—	4·0	—	—	—	—
—	—	—	28·3	—	2·8	—	—	—	84·0	7·1	4·0	—	—	—	—
—	—	—	5·7	0	—	—	—	—	84·0	18·6	7·0	—	—	—	—
—	—	—	22·3	0·015	2·6	1·43	3·4	0·57	87·1	6·4	4·5	—	—	—	—
—	—	—	41·3	0·026	5·5	—	—	—	87·0	8·6	5·7	—	—	—	—
—	—	—	33·6	0·066	3·4	1·12	4·3	0·62	90·8	11·7	6·9	—	—	—	—
—	—	—	58·8	0·098	5·2	1·27	2·8	0·66	84·4	6·5	5·4	—	—	—	—
—	—	—	38·4	0·093	3·2	0·89	1·55	0·58	80·9	8·9	7·0	—	—	—	—
—	—	—	60·0	0·085	5·5	0·98	2·14	0·53	85·4	7·4	5·5	—	—	—	—
—	—	—	30·0	0·099	3·0	—	—	—	81·5	10·0	6·7	—	—	—	—
—	—	—	35·5	0·216	3·1	2·47	4·16	1·86	82·1	9·2	7·2	—	—	—	—
—	—	—	30·0	0·163	3·1	0·89	1·45	0·92	—	—	—	—	—	—	—
—	—	—	46·3	—	5·0	0·81	2·77	0·30	—	—	—	—	—	—	—
—	—	—	56·0	0·117	4·8	—	—	—	—	—	—	—	—	—	—
—	—	—	40·0	0·156	3·1	—	—	—	—	—	—	—	—	—	—
—	—	—	17·5	—	—	—	—	—	—	—	—	—	—	—	—

—	—	—	—	—	—	—	Intravenous 50 cc. 5 % glucose.
—	—	—	—	—	—	—	Enema.
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	Enema.
—	—	—	—	—	—	—	Dog very weak and quiet.
—	—	—	—	—	—	5·200	Blood sugar estimations: before 0·25 %; after 0·40 % gluc.
—	—	—	—	—	—	—	Loewi's test negative.
—	—	—	36·2	32	10	5·000	Very weak and depressed.
—	—	—	—	—	—	—	Killed by chloroform.
—	—	—	—	—	—	—	
—	—	—	—	—	—	8·250	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	8·000	Operation I; partly depancreatised.
—	—	—	—	—	—	—	
—	—	—	—	—	—	7·750	Very active and hungry.
—	—	—	—	—	—	7·550	
—	—	—	—	—	—	—	
—	—	—	—	—	—	7·400	Abdominal skin incision slightly open
—	—	—	—	—	—	—	
—	—	—	—	—	—	7·200	
—	—	—	—	—	—	—	
—	—	—	—	—	—	7·000	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	Very well; abdominal skin incision healed.
—	—	—	—	—	—	6·750	P_2O_5 intake 11·0; P_2O_5 output 10·5.
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	6·750	
—	—	—	—	—	—	—	Removed from metabolism cage.
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	Removed from cage.
—	—	—	—	—	—	6·500	
—	—	—	—	—	—	—	
—	—	—	—	—	—	6·500	
—	—	37·8	74–80	—	—	—	In very good condition.
—	—	—	—	—	—	—	Thyroid administration: 1 g.
—	—	—	—	—	—	6·600	1·0
—	—	—	—	—	—	—	1·5
—	—	—	—	—	—	—	1·5
—	—	—	—	—	—	6·650	2·0
—	—	39·2	110	—	—	6·600	3·0
—	—	—	—	—	—	6·500	3·0
—	—	—	—	—	—	6·500	3·0; thyroid stopped.
—	—	—	—	—	—	—	
—	—	—	—	—	—	—	
—	—	—	—	—	—	6·350	
—	—	—	—	—	—	6·400	
—	—	—	—	—	—	6·450	
—	—	—	—	—	—	6·500	
—	—	—	—	—	—	—	Operation II; remnant of pancreas removed.
—	—	—	—	—	—	6·250	
—	—	—	—	—	—	—	
—	—	—	—	—	—	6·000	Very well, takes food readily.
—	—	—	—	—	—	—	
—	—	—	—	—	—	5·800	
—	—	—	—	—	—	—	Some pus discharge from scar of operation I.
—	—	—	—	—	—	5·650	Bright and active.
—	—	—	—	—	—	5·600	
—	—	—	—	—	—	—	Abdominal wound healed.
—	—	—	—	—	—	—	
—	—	—	—	—	—	5·350	Slight diarrhoea.
—	—	—	—	—	—	—	
—	—	—	—	—	—	5·200	Somewhat weak.
—	—	—	—	—	—	—	Died under anaesthetic. Only 4 hours urine was passed.